Earthworms, soil-aggregates and organic matter decomposition in agro-ecosytems in The Netherlands

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Earthworms, soil-aggregates and organic matter decomposition in agro-ecosytems in The Netherlands

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ABSTRACT

The relationships between earthworm populations, soil aggregate stability and soil organic matter dynamics were studied at an experimental farm in The Netherlands.

Arable land in general is not favourable for earthworm growth. In the Lovinkhoeve fields under conventional management earthworm populations were brought to the verge of extinction in a few years. Main causes are soil fumigation against nematodes and unfavourable food conditions. Organic matter inputs and N-contents of the organic materials are important aspects of food availability of earthworms, but also bacterial and protozoan biomass play a role. Natural stress, such as summer drought and winter frost, adds to the effects of management. Organic management with reduced pesticide use, however, strongly stimulates population development. Colonisation, especially stimulated in the presence of passive transport mechanisms can help population built up. Population parameters and growth conditions at the invaded plots further influence the speed of colonisation.

Correlation studies, combined with results of manipulation experiments show that earthworms increase resistance against slaking of field macro-aggregates. Fresh earthworm casts are very unstable, but their stability increases during ageing by several mechanisms. Drying makes casts much more stable than dried uningested field aggregates. Under specific conditions, fungal growth on the surface may stabilise wet casts, but the rearrangement of clay around particulate organic material seems to be the most important process for development of stability during ageing. Microbial activity either in the gut or after excretion may play an indirect role in connecting the organic material and the clay.

The organic matter included in stable aggregates formed under the influence of earthworms is much better protected against microbial breakdown during the lifetime of these aggregates than organic matter in stable aggregates formed by other mechanisms. In the presence of earthworms, stable aggregates are either formed at a higher rate or break down at a lower rate. Also, earthworm activity can lead to increased organic matter contents of macro-aggregates. Earthworm activity consequently leads to a concentration of organic matter in the larger aggregate fraction and to a higher organic matter retention capacity of the soil. Grinding soil to fragments smaller than 2 mm, as is often done in standard procedures, will strongly influence organic matter breakdown in incubation experiments in samples from soils with high earthworm activity. Therfore, these standard preteatment methods are unsuitable for fractionation and decomposition studies.

PhD Thesis. Department of Soil Science and Geology, Wageningen Agricultural University,
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NN08201, 1895

Stellingen

1 Het malen van grond mag geen standaardprocedure zijn bij decompositie-onderzoek.
Dit proefschrift

2 Regenwormen kunnen in de huidige Nederlandse akkerbouw de ploeg niet vervangen, wel aanvullen.
Dit proefschrift

3 Grondontsmetting tegen aaltjes met 1,3-dichloorpropeen dient, gezien de zeer nadelige invloed op het overige bodemleven, alleen in uiterste noodzaak te worden toegepast.
Dit proefschrift;
Special issue of Agriculture, Ecosystems and Environment 51 (1994): Soil ecology of conventional and integrated arable farming systems

4 Voor het onderzoek naar ontwikkeling van aggregaatstabiliteit onder verschillende condities is verregaande standaardisatie van methoden ongewenst.
Dit proefschrift

5 Het is lonend om akkers regenworm-vriendelijk te beheren.
Dit proefschrift

6 Regenwormen vormen een belangrijke bedreiging van het archeologisch erfgoed in de bodem.

7 Het wegblazen van dood blad uit plantsoenen en parkjes is niet alleen een energieverslindend en lawaaïg karwei, maar getuigt ook van weinig inzicht in natuurlijke kringloopprocessen.

8 In het pleidooi van de "School van De Wit" tot intensivering van landbouw uit het oogpunt van zuinig gebruik van hulpbronnen wordt de daarmee gepaard gaande locale vervuiling ten onrechte als een detailprobleem gezien.
CT de Wit, Agricultural Systems 40 (1992) 125-151

9 Het is zorgwekkend dat zelfs de Landbouwuniversiteit van Wageningen niet de breedte van het gebied onderkent, omvat door de term 'landbouw', gezien het plan die term uit haar naam te verwijderen.

10 De verkaveling van het onderzoek volgens de huidige vakgroepen- en cluster-indeling van de LUW verkleint in hoge mate de kans op de integratie van ecologische kennis in de landbouwwetenschappen, wat een voorwaarde is voor de ontwikkeling van milieuvriendelijke landbouwmethoden.

11 De bewering dat voor de gezonde ontwikkeling van een kind de invloed van de moeder allesbepalend is, is niet op verantwoorde wijze op wetenschappelijk onderzoek gebaseerd.
JC van Noordwijk-van Veen, Bio Nieuws 20, december 1994

12 Ouders zouden gebaat zijn bij een gemakkelijke methode om de biologische klok van kleine kinderen een beetje bij te stellen.

13 Onder invloed van de moderne mens wordt niet alleen de cultuur genivelleerd, maar, via erosie, ook de aardkorst zelf.

14 Het feit dat liefde een schaars goed is vormt de grootste bedreiging van en voor een individualistische maatschappij.
Milo Anstadt, NRC-handelsblad 23-1-1995

15 Geld maakt meer kapot dan ons lief is.

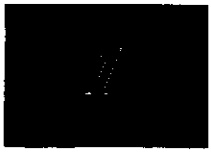
Stellingen behorend bij het proefschrift "Earthworms, soil-aggregates and organic matter decomposition in agro-ecosystems in The Netherlands".
JCY Marinissen, Wageningen, 20 februari 1995.

"It is a marvellous reflection
that the whole of the superficial mould has passed,
and will again pass, every few years
through the bodies of worms.
The plough is one of the most ancient
and most valuable of man's inventions;
but long before he existed
the land was in fact regularly ploughed
and still continues to be thus ploughed
by earthworms."

Darwin, 1881

Kaft: Oude gravure die al vele malen eerder gereproduceerd is. De oorsprong is mij onbekend, maar er zijn aanwijzingen die in de richting van Laurence Sterne (1713-1768) wijzen

Contents



General introduction

Introduction

The aim of the work reported in this thesis is to evaluate the importance of earthworm activity for the functioning of arable soils, in order to assess its possible contribution to sustainable agricultural management. Earthworm activity forms an important part of soil biological processes in sustainable agriculture. Before sketching their role in agriculture, I will present my view on 'sustainable agriculture'. The role of earthworms in agricultural systems will then be discussed with special emphasis on their biology and their influences on soil structure in general and on aggregate stability in particular. The research programme that this work was part of is subsequently described in more detail. This chapter ends with a general outline of the thesis.

Sustainable agriculture

Problems related to modern agriculture

In The Netherlands, agricultural production is high. The country has taken advantage of the 30 years of EU-policy that was aimed at increasing food security at low costs and to guarantee a fair income to farmers by price supports (De Wit 1988). Agricultural research too has long been aimed at maximizing production. In the high intensity agriculture, yield risks are prevented by high inputs. However, the public at large becomes more and more aware of the increasing environmental problems of this approach. Nutrients leak from soils into ground- and surface-water, causing health problems and affecting the natural flora and fauna. Biocides have already been found in drinking water, and antibiotics in manure affect soil microbes. Large scale farming includes structural changes of waterways, increased drainage, removal of line shaped landscape elements and levelling of natural differences in elevation, which all contributes to loss of cherished historical landscapes (De Wit 1988). The EU-policy has led to overproduction and high costs.

A discussion scheme

Discussions about sustainability of agriculture generally lack a theoretical framework from which criteria for the maximally tolerable amount of interference between agricultural areas and areas outside agriculture can be derived. Odum's (1971) suggestion to combine the opposing commands of agriculture on one hand and nature and the environment on the

other by spatial segregation of functional compartments is still very helpful. His concept, which was designed at a global scale, is presented in Fig. 1.1. Citing Odum: "Knowing the transfer coefficients that define the flow of energy and the movement of material and organisms between compartments, it should be possible to determine ..... rational limits for the size and capacity of each compartment". It will be necessary to adapt this model to different scales of time and space, depending on the questions dealt with. Decisions of minimal size and capacity of the compartments, and even of the type of compartments we want to select will remain political, but the framework will be a great help in the decision process. The fact that nature compartments can be affected by activities in production compartments, may limit the production intensity level acceptable in the latter.

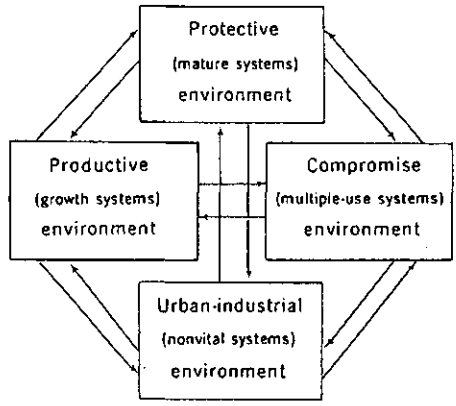


Fig. 1.1 Compartment model of the basic kinds of environment required by man. From Odum (1971)

Lessons from ecology

In natural ecosystems nutrient losses and pest impacts are reduced by the presence of complex food webs in a diversity of habitats. A food web consists of two subsystems that form two alternative pathways for energy flow and that are linked via the detritus: a pathway based on herbivorous consumption and a decomposition pathway. Interrelationships within food webs check the growth of populations of single potentially harmful species. However, pests can never be excluded, only balanced. In decomposer food webs the cycling of nutrients is modulated to the effect that losses are minimized.

To reduce environmental risks, agriculture could aim at following general ecological principles, to prevent diseases and nutrient losses, and/or apply specific biological techniques, such as biological control of pests. Agriculture involves many disturbances, and, as Odum stated already in 1971, agriculture is principally opposed to nature. However, agricultural systems could resemble 'eternally young' ecosystems, like tidal areas and savannas, that are governed by disturbances from outside. The recurrent disturbances prevent the ecosystems to develop into higher successional states. However, in 'eternally young' ecosystems all organisms are adapted to these disturbances, so still equilibrium on

a larger scale of time and space exists. It will be difficult to reach such equilibrium with planned and unplanned species in 'designed' agricultural systems on economically acceptable scales of time and space. However, small adjustments, such as introducing more spatial variability, e.g. by creating hedgerows, woodlots and wetlands may exert a relatively large stabilising influence on potentially harmful species in agricultural systems. The presence of hedgerows increased the number of bird and arthropod species (Mader 1988). Such small reserves could be a refugium and reinoculation source for species that cannot survive in the fields themselves, but can exert their influence in the fields from outside. Furthermore, hedgerows could serve as link between animal- and plant populations in nature reserves surrounding the agricultural areas. Mader did not give information on potentially harmful species living in hedgerows.

Implications and limitations of agriculture based on ecological principles

Agriculture based on ecological principles restricts many of the aims set in contemporary intensive production. The biological regulatory system needs "fuel" in the form of organic matter inputs. So, part of the crop must be 'sacrificed' to sustain a wide and complex herbivorous based food web that can balance pests. For the decomposer food web, inputs of dead organic matter should be high enough to sustain a biomass of decomposers, that can tighten nutrient cycling. The investments in foodwebs cost energy that is not directed to harvestable production. Therefore, in agricultural systems that utilise biological processes to regulate nutrient supply and to depress pests and diseases, net yields will be by definition lower than yields in technological agriculture at comparable levels of input.

Contemporary agriculture can be defined as 'technological'-oriented: the processes that in nature are exerted by biological agents are replaced by technical impacts. Problems that evolve can often not easily be solved with biological processes, and, hence, lead to more technological disturbances. These in their turn may harm the biological structure so much that new problems arise etc. Even in more biologically based agriculture technological disturbances cannot be avoided, however, because labour input will always be restricted by economic constraints. It will be a challenge to develop technologies that do as little harm to the biological functioning and potential of the system as possible.

The biological reactions following disturbances formed by necessary agricultural practice are complex, and depend partly on environmental circumstances, such as weather. This requires of farmers much more, but especially differently oriented knowledge than in contemporary agriculture.

This thesis aims at providing part of this knowledge. In this thesis, earthworms are seen as an important part of the agricultural food webs. Implications of agricultural management for their populations are taken into consideration. Furthermore, the important implications of earthworm activity on soil structure and soil organic matter dynamics are depicted.

The role of earthworms in the soil ecosystem

Earthworms as a group perform different functions in ecosystems. Earthworms (1) are part of the decomposer food web, (2) strongly influence soil structure and (3) influence organic matter dynamics both directly (via the food web) and indirectly (via the soil structure). However, not all species affect all aspects mentioned in the same way or to the same degree. Generally, for temperate areas, three ecological types of earthworms can be distinguished. The first group, the epigeics, are not real soil inhabitants, they live in concentrations of organic material, such as litter layers, woodlogs, compost heaps. The two types that influence the soil system most are the endogeics and anecics. Endogeics live in the soil, their food consists of more or less decomposed organic material, depending on species. To feed, they eat themselves through the soil. Some species can also sample food from the surface. Anecic species are the largest species. They sample their food at the soil surface, and live in deep, vertical, permanent burrows.

Earthworm ecology

Knowledge of the population ecology of earthworms is indispensable for quantifying the role of earthworms in the functioning of soil ecosystems. Furthermore, it may enable us to influence their activity. Which factors influence their life, how quickly does the population respond to various disturbances, what is regulating their numbers?

Earthworms are sensitive to the factors soil temperature, humidity, aeration/drainage status, clay percentage, organic matter content and pH (Diaz Cosin et al. 1994). Optimum temperature levels are 15-18°C for most species in The Netherlands (Evans and McLGuild 1948). Earthworms are affected by poor soil drainage, but until now no general quantitative relationships between soil water status and earthworm growth is known. Silty clay loam textures are generally optimal for earthworms (Gerard 1967). Soils which are high in organic matter because of high inputs of well palatable organic litter are favoured by earthworms. Palatability is probably the main factor through which crop type influences earthworm populations (Fayolle et al. 1994; Westernacher and Graff 1987), in addition to effects of crop type on the physical environment.

In the relatively young polders in The Netherlands, colonisation is still an important aspect of the population dynamics. Colonisation is not only important in young polder soils, however. Where earthworms have disappeared because of management, or where they have never developed because of adverse conditions (too wet, too little organic matter), earthworms may (re-)colonise the soil if management or conditions change. E.g. in the province of Limburg, the economic viability of a plan to combine badger activity and quarrying of sand and pebbles by area compartmentalisation depended on the colonisation speed of *L. terrestris* (the badgers' main food source) into the restored land. Not much data

on colonisation of this species are known, but unfortunately, this is the most slow coloniser of the earthworms of The Netherlands. More research is needed before plans like this can be executed.

Earthworms as part of the food web

Earthworm biomass can make up a large part of the total decomposer food web biomass, thereby representing a large pool of nutrients (Marinissen and De Ruiter 1993). They form a link between the decomposer food web and the above-ground food web, because they are an important food source for many birds and small mammals. Earthworms consume large amounts of both soil and dead plant remains. Most species preferentially ingest finer fractions of the soil rich in organic matter together with particulate organic matter (Piearce 1978). By this, particle size of dead plant remains is reduced, so that more of the material becomes available to smaller primary decomposers. Earthworms are not purely primary decomposers: microbes and protozoa form an important part of their diet (Miles 1963). The contribution of earthworms to total C-respiration can be high, especially when the indirect effects on microbes are taken into account: up to 440 kg C ha^{-1} $year^{-1}$ (Marinissen and De Ruiter 1993). The N-flux through earthworm activity has been estimated by different authors. Parmelee and Crossley (1988) reported a flux of 60 kg N/ha/yr, which represents as much as five times the standing stock of N present in the soil. Marinissen and De Ruiter (1993) estimated N-mineralisation by earthworms in arable soil to vary between 5 and 30 kg N/ha/yr, or 3-20% of the total N-mineralisation measured in the field. Hameed et al. (1994) found very rapid transfer of N excreted by earthworms to plants: within 48 days 80% of ingested 15N was excreted again, and 24% was taken up by plants (15°C, field and lab).

Earthworm influence on soil structure and organic matter dynamics

Earthworms contribute to soil structure formation in different ways. On the one hand they form channels, that connect the surface to deeper soil layers, on the other hand their casting activity leads to the formation of crumby, aggregated soil structure. By channel formation the total porosity can be increased, and the relative amount of macropores is enlarged. Pore systems formed by earthworms in different tilled systems have been studied extensively by Ehlers (1975), Edwards (1975) and Edwards et al. (1990). Channels influence the aeration status of the soil also in a wet state (Marinissen, unpubl). Channels that are open to the surface facilitate the entrance of small structure-following animals into the soil (Malinda et al, cited by Oades 1993, Marinissen and Bok 1988). The channels may function as drainage ducts during rain storms (Edwards et al. 1989; Bouma 1981, Bouma et al. 1982). This may lead to N-losses from surface applied fertiliser. However, N present through mineralisation in the soil matrix is conserved by the presence of channels because the water

front by-passes the matrix (Elliott and Coleman 1988; Ernst and Tolsma 1989; Sollins 1989). According to Munyankusi et al. (1994) the effect of channels is often overrated, because they easily get clogged at the soil surface. Trampling by cattle indeed destroyed channels in the surface soils (Ligthart et al. in press) but inhabited channels will be soon be reopened by the worm living in it.

The soil structure created under well drained conditions by earthworms is characterised by rounded aggregates with heterogeneous compound packing voids between them (Marinissen and Bok 1988). Aggregated soils have a higher percentage of macro-pores and, possibly, a favourable balance between water- and air-filled pores for root growth and microbial activity. In the presence of earthworms more stable soil aggregates are present than without them (a.o. Ketterings et al. in press). Willems et al. (1993) found that in undisturbed field soil structure, that was at least partly induced by earthworm activity, N-mineralisation was stimulated more than in sieved soil.

The dynamics of organic matter is influenced by earthworms both through their feeding activities and through their influence on soil structure. Within the food web earthworms consume and partly digest organic material, and directly stimulate microbial activity by their grazing. The aggregated soil structure created by earthworms offers better conditions for aerobic decomposition (Hoeksema and Jongerius 1959). The limited available evidence (Marinissen and Kroesbergen, unpubl results; Joschko et al. 1989) indicates that earthworm casts of temperate area species have higher porosity than field aggregates. Casts from some tropical species have been reported to be much more compact, or even coated with a crust (Blanchart et al. 1993), that may explain reduced decomposition in these casts. Furthermore, earthworms translocate and fragment plant litter. The retranslocation of part of the organic material into (stable) aggregates might considerably influence organic matter dynamics (Ladd et al. 1993), as will be described in part of this thesis.

Aggregate stability

In this thesis the main aspect of the effects of earthworms on soil structure studied is aggregate stability. Earthworm influence on formation and stability of aggregates is a little studied field, while other aspects of the influence of earthworms on soil structure have been treated extensively by other authors. Another important reason to study aggregates is that soil aggregates directly link soil structure and organic matter dynamics.

Aggregate stability is not a simple, easily defined characteristic of the soil. It can be defined as the resistance to a certain type of disturbance. The type of binding agents needed for resistance against specific stresses may vary with the type of disturbance applied. It logically follows that aggregate stability against different treatments can be very variable: the order in which aggregates from differently treated fields are resistant to mechanical

loads can be quite different from that involving resistance against rapid wetting. Together with different types of stability, different classes of methods can be used to measure aggregate stability: breaking force measurements (Dexter et al. 1984), deformation depending on water status (Atterberg 1910), resistance against quick wetting (Loch and Smith 1986), strength against sieving in a wet state (Beare et al. 1994b), or combinations of the latter two disturbances (Elliott 1986), or resistance against dispersion of clay, either in salt solutions (Dettman and Emerson 1959), or water (Pojasok and Kay 1990). Differences in pretreatment and procedures used will influence the outcome of the measurements. A comparison of the different types of measurements is outside the scope of this thesis, and good reviews (Matkin and Smart 1987; Molope et al. 1985b) and comparative studies (Beare and Bruce 1993) are available.

The many different types of stability and methods to quantify it, make it difficult to compare results from different studies. However, it would not be wise to strive for one uniform method, because no single method fits all soils and answers all questions. E.g., the strength exerted on the aggregate during wet sieving depends on the combination of sieving time, frequency and amplitude. Standardisation would reduce the range of applicability of such a method. A 'harsh' method may lead to 0% stability in unstable soils, which, however, may differ in stability when sieved in a more gentle way. On the other hand, gentle sieving may lead to 100% stability in more stable soils without revealing possible differences. As none of the stability measurements is directly related to real-field situations, trends and differences between treatments are more important than the actual values measured. Boersma and Kooistra (1994) found greater internal transport of clay in soils with a low stability measured with a gentle wet-sieving technique (Marinissen 1994), and less internal transport in the field with highest stability. Descriptions of the methodology used should be very clear, however, so as to enable the reader to judge the harshness of one method relative to another for the same type of measurement.

The Dutch Programme on Soil Ecology of Arable Farming Systems

Description of the experiment

Most of the work described in this thesis was performed as part of the Dutch Programme on Soil Ecology of Arable Farming Systems, carried out at the Lovinkhoeve experimental farm at Marknesse, Noordoost Polder (see Fig. 1.2.). This polder was reclaimed recently (1942). In this programme, 'conventional' agricultural management was compared to a form of 'integrated' management. The 'integrated' management was aimed at using biological processes to get a better nutrient use efficiency, sustained improvement of the soil structure and effective pest and weed control. The farm management in 'integrated' differed from

'conventional' in the following aspects (Brussaard et al. 1988):

- reduced nutrient inputs
- a larger part of the nutrient inputs through organic amendments
- reduced (shallower) tillage
- reduced use of biocides

Application of biocides could be reduced because fewer pests were expected at lower nutrient levels. Preventive soil fumigation could be omitted in the 4 year-rotation using resistant potato-varieties. Some specific biological control organisms were used where appropriate, such as inoculation of potato-tubers with a fungus suppressing *Rhizoctonia solani*. Weeds were largely controlled mechanically. The reduced nutrient inputs reduced the sensitivity to pests, but also improved the nutrient use efficiency. Lower costs should balance the lower returns from reduced yields, which was aimed at 80-90% of that under conventional management. The reduced tillage depth (15 cm instead of 25 cm) was chosen to reduce disturbance of the many species of the food web living in the top soil layers that are susceptible to tillage. Details on the management are given by Brussaard et al. (1988) and Lebbink et al. (1994). Details on the soil and the reclamation and management history of the plot are given by Kooistra et al. (1989).

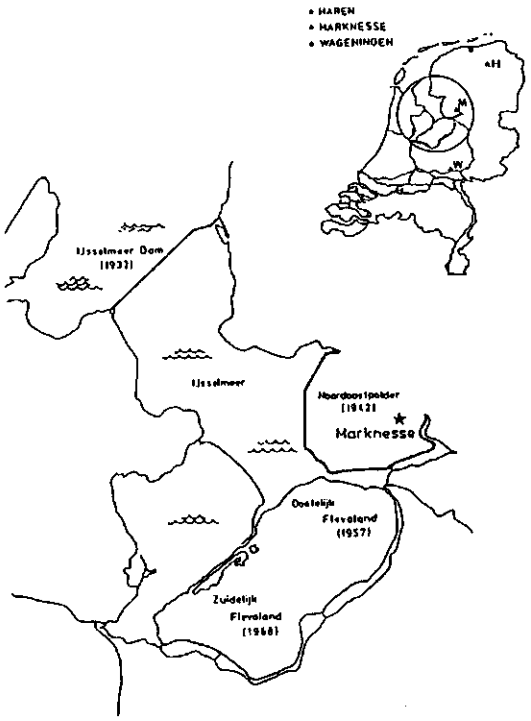


Fig. 1.2. Location at the experimental farm Lovinkhoeve, The Netherlands. From Lebbink et al, 1994

The experiment at the Lovinkhoeve was laid out on fields that had received 35 years of different organic matter management. One block (A) had a rotation including leys, and a large part of the nutrients were derived from organic sources (manure, crop remains). The other block (B) did not have a ley in the rotation and only received fertiliser. The new management systems (conventional and integrated) were laid out again in two blocks perpendicular to the old treatments. In this way 4 combinations came about: Conv-A, Conv-B, Int-A and Int-B.

Outline of the thesis

The first three chapters and part of chapter 4 describe earthworm population dynamics in the Lovinkhoeve fields. Because the polder soils in the study area were reclaimed only recently, colonisation by earthworm is still ongoing. Chapter 2.1 describes the colonisation pattern and processes in the Lovinkhoeve fields. More general knowledge of colonisation by earthworms, or specific groups of earthworms, is provided by chapter 2.2, where a model is discussed that aims at predicting general colonisation patterns for different earthworm species. Population dynamics of earthworms in the fields under different management are described in chapter 3 and in part of chapter 4. Chapter 3 contains a factorial analysis of the environmental factors influencing population density.

Changes in aggregate stability in the fields of the Lovinkhoeve over the years and the role of earthworms in that development are described in chapter 4. Aggregate stability over one season was related to earthworm numbers in two fields with different management. Fields from other parts of the Lovinkhoeve, differing in only one aspect from conventional agriculture, were considered, too. In chapter 5 mechanisms for aggregate stabilisation by earthworms are described as found in a laboratory experiment comparing earthworm casts with artificial casts made from two different types of soil. Chapter 6 deals with the behaviour of clay in wet casts of different types of soil and discusses the implications for organic matter dynamics. In chapter 7 preliminary results of experiments on the relationships between aggregate stability and decomposition of organic matter in the Lovinkhoeve field as influenced by earthworms are discussed. In Chapter 8, the general discussion, the results of this research are brought together in a general theory on the influences of agricultural management on the earthworm activity and on the mechanisms by which earthworm activity affects aggregate stability and organic matter retention potential of the soil.

Colonisation potential of earthworms into new habitats

2.1

Colonisation of arable fields by earthworms in a newly reclaimed polder in The Netherlands. Preliminary results

JCY Marinissen (1991) In: GK Veeresh, D Rajagopal and CA Viraktamath: Advances in management and conservation of soil fauna, proceedings 10th Int. Soil Zoology Colloquium, Bangalore, India 1988 Oxford and IBH Publishing Co., New Delhi

Summary

Colonisation of an arable field by earthworms was studied in a newly reclaimed Dutch polder. The rates of spread of recently introduced populations and populations established 30 years ago, both on an experimental farm near Marknesse in the "Noord Oost Polder" are shown. Factors influencing colonisation of arable fields, related to population dynamics and passive dispersal, are discussed. From the results, it appeared that in arable soil active dispersal is slower than in grassland situations, while dispersal by mud transport on tractor wheels can be considerable.

Introduction

Over the last few decades, intensive agricultural practices in The Netherlands have probably reduced earthworm populations in arable land. Nowadays, reduced input production is the subject of an increasing research effort. One way to minimise inputs is to reduce tillage, using natural processes of soil structure conditioning. To help this process, (re)colonisation of earthworms should be considered.

Little is known about mechanisms involved in the colonisation by earthworms. Mostly transport to new areas can be either by the activity of the animals themselves (active dispersal) or through the action of other agents (passive dispersal). Schwert (1980) described two ways of passive dispersal, transport by stream drift (along the borders of rivers), or by other animals. Cattle can be effective in spreading earthworm cocoons in the mud on their hooves (JP Curry, pers. comm.). Schwert only described one way of active dispersal only, by mass emergence occurring under stress conditions. However, under normal conditions, active dispersal can also be expected to be a result of the random movement of earthworms through the soil, a behaviour that is necessary for endogeic species to feed themselves. If the random movement of individuals is involved, then the rate of spread of the population border depends on both population size and the activity pattern of the individuals (Williamson and Brown 1986).

It is likely that factors involved in determining the rate of spread of earthworm populations (population growth, individual rate of both active and passive dispersal) may be different under varying conditions. In grassland plots population growth can be high, and passive dispersal is restricted to small amounts of soil in cattle hooves. In grasslands in

newly reclaimed polder soils colonisation proceeded with a rate of approximately 8 meters per year for *Aporrectodea caliginosa* and *Lumbricus rubellus* (Hoogerkamp et al. 1983). Curry and Boyle (1987) studied the establishment of earthworm populations in reclaimed cut-over peat. They found a dispersal rate from inoculum sods of at least 2.5 m $year^{-1}$ for *A. caliginosa*, and 10 m $year^{-1}$ for *L. rubellus*.

In arable fields, active colonisation can be expected to be much slower, because of less favourable conditions for earthworms, resulting in lower population densities. On the other hand, individual movement may be stimulated by lower food availability (Martin 1982). Passive dispersal by soil transport with tractor wheels may also take place under arable conditions. This type of spread would cause a skewed distribution in the direction of the main tillage operations, and would take place mainly during tillage carried out under relatively wet conditions.

From the foregoing can be concluded that, to be able to predict colonisation rates under different conditions, it is necessary to measure parameters of both active and passive dispersal. In this study, measurements of individual dispersion range and an estimation of passive transport rate are presented.

Methods

Site description

The site where the research was carried out is the "Lovinkhoeve" experimental farm in the Noord Oost Polder, which was drained in 1943. The soil is a calcareous sandy loam soil (Brussaard at al. 1988). Different parts of the farm received different treatments (see Fig. 2.1.1). South-East of the farm the fields have been under pasture since 1943, but are now used as arable land. South to Southwest of the farm a plot has been treated with different types of fertiliser. Block A received farm yard manure, inorganic fertiliser and green manure, block B received only inorganic fertiliser, while block C received both green-manuring and inorganic fertiliser. Since 1985 the fields of this block have been split into blocks with conventional and integrated crop production. The integrated crop production is characterised by lower inputs of inorganic fertiliser and biocides, more use of organic manuring and reduced tillage (for details see Brussaard et al. 1988). South of these blocks a long-term tillage experiment has been established, where minimum and conventional tillage methods are compared.

Fig. 2.1.1 illustrates the distribution of the earthworm population as found during 1987 (in June and October). Population density and species diversity were the highest in the most northern plot which was a grassland until recently. The minimum tillage plot, farther away from the farm yard, did not have earthworms. In block B, which received only inorganic

fertiliser, earthworms were absent or rare, only in the integrated part of block B some *L. rubellus* occurred. In the southernmost field of block A only one species (*L. rubellus*) was present, while in the field closer to the farm the endogeic species *A. caliginosa* and *A. rosea* were also present.

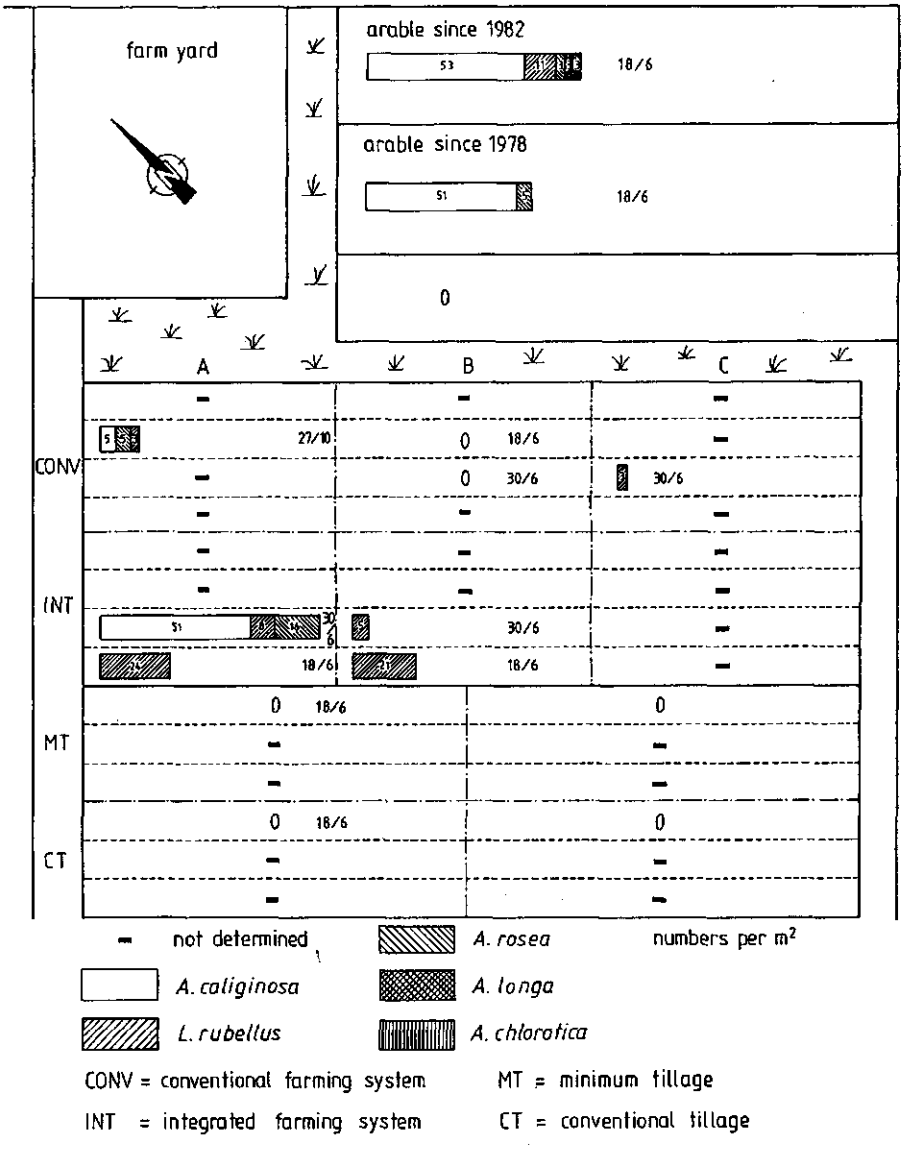


Fig. 2.2.1 Map of Lovinkhoeve experimental farm, including earthworm distribution over the farm

Experimental setup

To estimate active and passive dispersal, two experiments were carried out. For the measurement of active dispersal, earthworms were inoculated in a minimum tillage plot in April 1987. One hundred and twenty individuals, both adults and sub-adults of *A. caliginosa* were laid on the surface of a 1 m^2 quadrant, that was free from tractor tracks. There were four replicates. The plots were covered with a thin layer of compost to prevent the night frost from harming the worms. The compost was removed after one week, but some remained in small cracks in the upper soil. When necessary, the plots were watered to keep the soil moist enough for earthworm activity. This watering was only necessary in June; the summer of 1987 was extremely wet. On October 6, survival and spread was assessed by sampling one 20x20x20 cm^3 subplot at each inoculation site and two more, one and two meters away from it. Because of the destructive nature of the sampling, only two inoculation plots and adjacent areas were sampled. The soil was washed away over a sieve, and from the debris earthworms and their cocoons were collected. The period from April to October covers the main part of the active period of the earthworms, cold and frost limiting activity during the rest of the year. Also harvest (mid-October) and subsequent tillage disturbs the active dispersal pattern.

In a second experiment, passive dispersal of cocoons by tractor wheels was simulated using small (2mm) glass beads. In a plot of roughly the size of a tractor wheel imprint over one revolution (30x500 cm), 2900 coloured glass beads were pushed into the soil with thin wooden pins to a depth of 1.5, 3.0 and 4.5 cm, each depth represented by a different colour. These depths were chosen because the ridges on the tractor wheel were 5 cm high, and it was not expected that beads buried deeper would be in reach of the wheel. There were five replicates. After a weekend of heavy rainfall, when the soil was sticky, a tractor was driven with the right wheels over the plot and over 65 m of bare soil with normal ploughing speed. Subsequently, the soil adhering to the right wheels was sampled for the remaining beads. All the soil was removed from the wheels before the tractor was allowed to drive to the next replicate (a distance of about 80 m, enough to fill the wheels with soil before driving over the glass bead plot again). In the track the right wheels made, all clods that fell from the wheels were sampled in intervals of 5 meter from the inoculum point. The clods were weighed and the number of beads in them determined by a washing and sieving procedure. Also in the first 25 m of each track after the inoculum plot, every 5 m a random sample of 20x20x7 cm^3 was taken, and the number of beads in it measured. In the inoculation plot, three such samples were taken to determine the number of beads that were left over after the passage of the wheels.

Results

The inoculation experiment in the minimum tillage plot showed a good survival of the introduced earthworms. The results of the counts are given in table 2.1.1. *L. rubellus* had appeared in the plots, although it was not introduced deliberately. Possibly *L. rubellus*

Table 2.1.1 Number of earthworms found in inoculation plots adjacent areas (total of two replicates)

	Number in sub plots of size 20 x 20 x 20 cm^3 in		
	Inoculation plot	1 m further away from inoculated plot	2 m further away from inoculated plot
A. caliginosa			
worms	29	1	0
cocoons	25	2	1
L. rubellus			
worms	2	0	2
cocoons	16	41	16

cocoons were introduced with the compost cover, which was not completely removed. From the presence of earthworm casts on the surface, it became clear that the earthworms had not spread over more than 2-3 m from the inoculation plot. From table 2.1.1 it appears that *L. rubellus* has spread over a distance of 2 m, while *A. caliginosa* did only incidentally come much farther than 1 m.

The results of the passive dispersal experiment are presented in Figs. 2.1.2 and 2.1.3, and table 2.1.2. The distribution of glass beads over the field in the clods (Fig.2.1.2) shows a regular pattern for the first 35 m, then decreases sharply. In the wheel track itself (Fig.2.1.3) the highest amounts of beads were found. Most were recovered in the first 15 m, followed by a sharp decline. The balance of numbers of beads recovered is given in Table 2.1.2. Of all the beads, on the average 50.2 % was removed from the plot where they were applied. More beads that were buried superficially (yellow) were transported than those buried deeper. After 65 m, only few beads (3.1 %) were still adhered to the wheels. In case of superficially and deepest buried beads, 8.8 and 21.4 % respectively of the beads were not recovered.

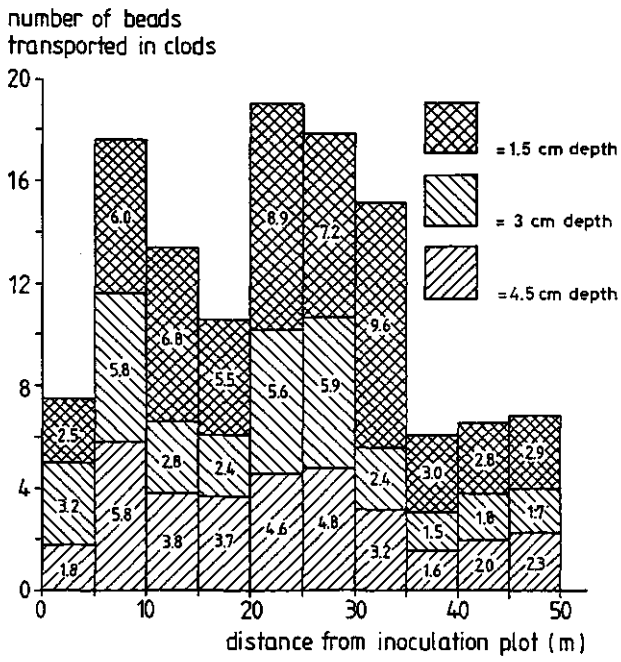


Fig. 2.1.2 Number of beads in clods transported by the tractor wheel, in relation to distance from the inoculation plot. Mean of 5 replicates

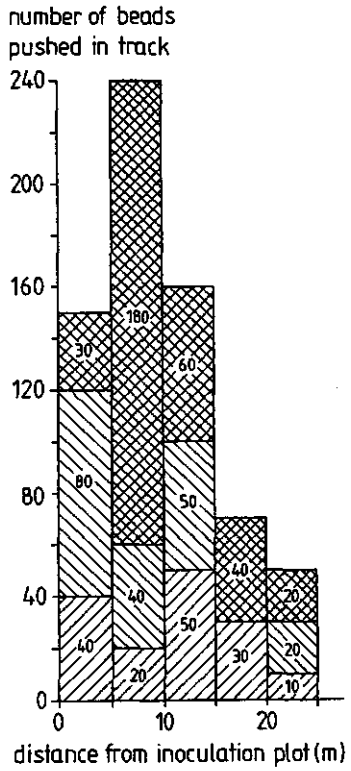


Fig. 2.1.3 Number of beads found in the track of the tractor wheels, in relation to distance from the inoculation plot. Hatching as in Fig. 2.1.2

Discussion and conclusions

In 1959 earthworms were present in the meadow near the farm (van Rhee, unpubl. results), and rare in at least parts of block A (pers. comm. Doeksen, 1988). Nowadays, earthworms can be found in the most southern parts of block A (Fig. 2.1.1). They are scarce in the northern part of this block, which may be attributed to the present conventional treatment, in which nematicides have been used. Block B can be expected to have been uniformly unfit for earthworms, having received only inorganic fertiliser for 30 years. Under the new integrated management in part of block B it can be expected that conditions for earthworm survival have become much better. The presence of *L. rubellus* and in autumn 1987 also of *A. caliginosa* (unpublished results) points to this. The absence of earthworms in the

minimum tillage plot is not as could be expected from data from other tillage experiments (Westmaas research group 1984; Ehlers 1975). The inoculum-experiment showed that *A. caliginosa* was able to survive and reproduce well in the minimum tillage plot during the summer 1987.

Table 2.1.2 Total balance of beads inoculated and transported (Mean of five replicatesd)

	Yellow (1.5 cm)	Bleu (3 cm)	Red 4.5 cm	Total
Inoculated	960	960	960	2880
Left over after passage of wheel	415	527.5	492.5	1435
Transported in clods over 50 m	55.2	33.1	33.6	121.9
Pushed in track over 25 m	330	190	150	670
Over in soil adhered to tractor wheel	37.2	28	24.8	90
Estimated in track 25-65 m	56.2	52.5	53.4	162.1
Not accounted for	85.4	128.9	205.7	420

It seems likely that a colonisation has taken place which proceeded from the farm yard over the grassland plots, and from thereon southward over the organically treated block A. During the survey for this research, the colonisation border seemed to have just reached the minimum tillage plot.

From the field distribution the over-all colonisation rate would be approximately 175 m in 35 year, this is 5 m per year. This rate is lower than that found in grassland by Hoogerkamp et al. (1983). Here colonisation proceeded with a rate of approximately 8 meters per year for *A. caliginosa* and *L. rubellus*. Grassland without worms was characterised by a 2.5-4 cm thick organic layer, which provided ample amount of food to the pioneer population. Because the organic layer disappears in the centre of the earthworm plot, highest population growth can be expected at the population border, causing a relatively high rate of dispersal, assuming that the individual activity pattern is not changed under those conditions. Curry and Boyle (1987) studied the establishment of earthworm populations in reclaimed cut-over peat. They found a dispersal from inoculum sods of at least 2.5 m. yr^{-1} for *A. caliginosa*, and 10 m. yr^{-1} for *L. rubellus*, also under relatively favourable grassland conditions. The value found for the overall dispersion in this study is rather high compared to this values.

In the inoculation experiment, a rate of only 2 m was found for active dispersal of *A. caliginosa* for the main part of its activity period. It therefore is likely that in the colonisation of block A also passive dispersal has been important. During wet years, earthworms of the species *L. rubellus* and *A. caliginosa* tend to remain at shallow depths,

and also lay cocoons in this layer (unpublished results). Also in wet years, transport of soil by tractor wheels can be considerable. During harvest after the wet summer of 1987, tractor wheels carried a steady load of over 30 kg of soil after driving over a field in block A, which was found to contain 260 cocoons and 45 live earthworms, including some adults. On the other hand, farmers try to restrict tillage operations to dry periods, during which both the number of cocoons in the surface layer and the amount of soil transported with the wheel is low.

The survival of cocoons transported by the wheel depends on several factors. The direct damage during uptake by the wheel is probably rather small, only 5 damaged cocoons could be found in the soil from the wheel at harvest 1987. Cocoons pushed into the track, which could represent half of the cocoons transported according to the passive dispersal experiment, can be expected to be severely damaged by the pressure. Those transported in the clods are dispersed over the soil surface and are in summer at risk under dry conditions, and in winter during frost. But if the cocoons are transported during harvest, often the soil is ploughed soon afterwards, increasing the chance that the cocoons are brought to safer depths before drying out is liable to occur.

Most of the cocoons on the tractor wheels will be transported over relatively small distances (15-25 m.), as could be concluded from the dispersal experiment with the glass beads. However, small numbers of glass beads were spread with the clods that fall from the wheels at much greater distances, and can be expected to be spread even further than 65 m. So, in those years where cultivations are done under wet conditions, especially in the autumn, pioneer colonies may be started further from the colonisation border than can be expected from natural dispersion. Within the field, the direction of tillage operations is SW-NE and vice versa, influencing the colonisation of block B from block A very much. This is reflected by the relatively quick colonisation of *L. rubellus* into block B. In the North-South direction passive dispersal is not a regular process, but one that is unpredictable to a certain level, as the order in which the farmer works the fields is different throughout the year from year to year.

Future plans are to fit the data from this study, and literature data for other species into the model of dispersal that has been described by Van den Bosch et al. (1992).

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2.2

Colonisation of new habitats by earthworms

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Abstract

In this paper a simple model is used to study the dispersal of earthworm populations into new habitats. Simple models do not describe processes accurately, but can help gain insight into the functioning of ecosystems or processes in ecosystems. Using information on reproduction, survival and dispersal at the level of the individual, the velocity of earthworm population expansion was calculated. Dispersal of earthworms can be active or passive. The parameters of active and passive dispersal were calculated from field experiments done in one of the Dutch polders. Parameters of reproduction and survival were estimated from published data. The effects of processes at the individual level on the velocity of population expansion were studied for two species (*Aporrectodea caliginosa* and *Lumbricus rubellus*). The model shows that passive transport has a major influence on the velocity of population expansion, which is strongly increased even if this transport involves only a very small part of the population. At a high level of passive transport, however, death induced by this mode of dispersal can have a negative influence on population expansion. In the discussion it is indicated that optimising growth conditions of the earthworms might be the easiest way to promote population expansion. However, promoting dispersal by passive mechanisms can also be very important.

Introduction

Earthworms enhance the incorporation of organic matter into soils and the formation of soil structure (Lee 1985). Colonisation of earthworms is therefore useful when restoring mining spots and reclaiming new land (Brun et al. 1987). Data regarding dispersal on a field-scale have been reported for grassland situations. These data mainly concern population dispersal, while data on dispersal processes at the individual level are lacking. Earthworms can disperse actively and passively. Active dispersal involves movement through the soil in search for food (Martin 1982). Some species (*L. terrestris* and *A. caliginosa*) also move over the surface (Bouché 1976). Passive dispersal mechanisms for earthworms include transport with eroded soil (Schwert 1980), with water (Frenot in press) and by birds (Meijer 1972). In arable land, transport of soil by agricultural machinery also may play a role in earthworm dispersal.

Models help to understand the behaviour of biological systems. Two types of models can be distinguished: "realistic" models, mostly very detailed, and "strategic" models (Levins 1966). Simple, strategic models are valuable in understanding main mechanisms and in evaluating the influence of management practices on the system, as has been shown by Scheffer (1989, 1990, 1991). In this study, we present a simple model, adapted to earthworm behaviour, in analogy to the model by Van den Bosch et al. (1990). The model describes the velocity of spatial population expansion of various earthworm species. Furthermore, it can be used to investigate the relative importance of various factors at the individual level affecting the velocity of population expansion.

Demographic parameters were estimated from data available in the literature. Experiments were performed to quantify both active and passive dispersal of individual earthworms. Data on population dispersal patterns in arable land, and literature data on population dispersal in grassland, served as tests of the model.

Methods

The model

Spatial expansion of a population into new territory results from reproduction and dispersal of individual members of the population. The relevant characteristics at the individual level are:

I. *Demographic characteristics*: the age-specific survival, $L(a)$, and the age specific fertility, $m(a)$.

II. *Dispersal characteristics*. The dispersal density, $D^*(a,x,\zeta \mid \text{alive})$, is defined as the probability that an individual born at position ζ, if still alive at age a, is living at x. This dispersal density is assumed to be rotationally symmetric. We do not assume Brownian motion as for example the diffusion model of Skellam (1951).

The model can be viewed as a bookkeeping system, keeping record of the age and place of all individuals in the population. Assume, for example, that the invasion is started with one newborn at position $\zeta=(0,0)$. The demographic characteristics determine how long this individual will live and when it gives birth. The dispersal characteristics determine where it will give birth. For every newborn the model registers time and place of birth. The total of all these records indicates, at any moment, how many individuals are living and where they are located. This type of bookkeeping system can be described mathematically (Diekmann 1978, 1979; Thieme 1977, 1979; Van den Bosch et al. 1990). The mathematical description is a spatial variant of the well-known Lotka equation for the growth of an age-structured population (see e.g. Roughgarden 1979).

The general model was studied extensively by Diekmann (1978, 1979), Thieme (1977, 1979) and Van den Bosch et al. (1990). Only few assumptions on the shape of $L(a)$, $m(a)$ and $D^*(a,x,\zeta \mid \text{alive})$ were required. In the present context these assumptions are met and for details we refer to the original papers. After a phase of population build up, a population wave develops. Travelling-wave solutions of the model can be visualised as wave-shaped population density profiles, travelling with a constant velocity, C, through space (Fig. 2.2.1). Van den Bosch et al. (1990) showed that the velocity of spatial population expansion, C, can be calculated from demographic and dispersal characteristics of a species, using an explicit and simple approximation formula. For a species that disperses throughout its life, such as earthworms, the formula is:

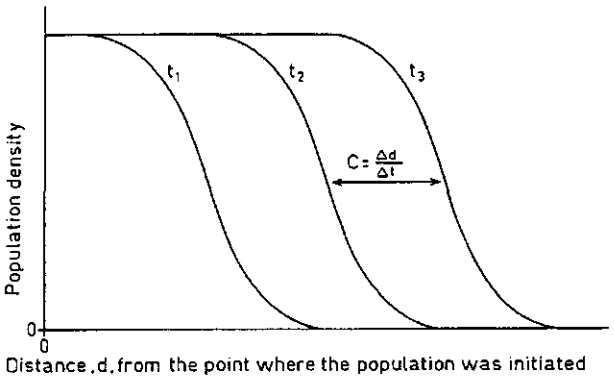

Fig.2.2.1 Wave of advance of a population. The population is initiated at point 0 on the x-axis. Population density profiles are given for successive times, t_i, at equal time intervals. The velocity of population expansion equals the distance between two population profiles derived by the time interval.

$$C \approx \frac{\sigma}{\mu}\sqrt{2lnR_0}\,(1+\frac{1}{12}\gamma lnR_0) \qquad (1)$$

The parameters in this equation are:

A. *Reproductive parameters*: μ and R_0. μ is the mean age (in years) at which an individual reproduces, R_0 is the net-reproduction (number of offspring per individual, in a density independent situation). It is biologically obvious that a species which start to reproduce at an earlier age has a larger velocity of population expansion. This is reflected in the inverse relation between μ and C in Eq. 1 Also, the larger the number of offspring, the larger the velocity of population expansion, see Eq. 1.

B. *Dispersal parameters*: σ and γ. σ is the standard deviation of the dispersal density. This parameter measures the average distance travelled by an individual. The larger this average distance, the larger the velocity of population expansion, as reflected in Eq. 1. γ is the kurtosis of the dispersal density. This parameter is a measure for the shape of the dispersal density. The kurtosis of the normal density, for example, is 0. The double exponential distribution, which has "thicker tails", has a kurtosis of three. A "thicker tail"

causes a larger velocity of spatial population expansion, as is reflected in Eq. 1.

To apply Eq. 1 to earthworm dispersal patterns some of the parameters were modified as follows.

R_0: The net-reproduction is modified to account for mortality associated with passive dispersal. In the absence of passive dispersal an earthworm has a net reproduction R_0^*. If a fraction F of the earthworms is transported passively, of which a fraction δ survives this mode of transportation, the final net-reproduction is

$$R_0 = (1-(1-\delta)F)\ R_0^* \tag{2}$$

μ: As an approximation we assume that individuals hatch in spring (Evans and Guild 1948; Cotton and Curry 1980). For first six months the individuals are juveniles. Reproduction thus takes place in the second half of the year. During winters all individuals, except cocoons, die. From this we find

$$\mu \approx 0.75 \tag{3}$$

σ^2: If the variance of the active dispersal is σ_A^2, and that of the passive dispersal σ_P^2, the variance of the total dispersal is equal to:

$$\sigma^2 = (1 - F)\sigma_A^2 + F\ \sigma_P^2 \tag{4}$$

γ: Little is known about the activity pattern of earthworms in the soil. The most reasonable assumption is that they perform a random walk. This means that at every moment the dispersal density of the active dispersal follows a normal distribution, i.e. the kurtosis is 0. For passive dispersal by tractor wheels, a constant probability per unit of time has been assumed for an individual to fall off the wheel. This results in an exponentially distributed density in the direction of tractor movement. The kurtosis of the exponential distribution is 3. For the total dispersal we then have

$$\gamma = 3\ F \tag{5}$$

Combining Eq. 1 and 3 through 5 yields

$$C \approx 1.3\ \sigma_A \sqrt{(1-F) + F(\sigma_P/\sigma_A)^2}\ \sqrt{2lnR_0}\ (1 + 0.25FlnR_0) \tag{6}$$

where R_0 is given in Eq. 2

Parameter estimation

Reproduction parameter: R_0. The net-reproduction, R_0, can be estimated from: (a) the number of cocoons produced per adult; (b) the percentage of successful hatchings and (c) the probability that a juvenile becomes a reproductive adult. The number of cocoons produced by different species have been studied by Evans and Guild (1948). Hatching success of *L. rubellus* and *A. caliginosa* is estimated from our own unpublished results as being 70%. The chance of a juvenile reaching the reproductive stage in arable land is approximately 25% (Boström 1988) for both species studied.

Active dispersal parameter: σ_A. An introduction experiment was started in 1987 at a field at the Lovinkhoeve experimental farm, Dutch North East Polder (established in 1937), where earthworms had not yet invaded. Inoculation plots were established outside tractor tracks, so that only active dispersal took place. The spatial distribution of worms was determined one year after inoculation using manure traps (Lofs-Holmin 1979). Due to dry soil conditions during trapping this method yielded data on *L. rubellus* only.

For grassland situations, we measured dispersal of earthworms in a non-grazed newly sown ley in Nagele in the Noord Oost Polder, 1 year after inoculation of a mixture of *L. rubellus*, *A. caliginosa* and *Aporrectodea longa*. During inoculation, 200 worms were evenly distributed in each of four 1-m^2 plots in October 1987. In November 1988, from each inoculation plot, a linear sampling was carried out, on which at every meter a sample of 25x25x25 cm was sorted by hand, up to a distance of 10 m. From the resulting distance-frequency tables σ_A can be calculated according to Steel and Torrie (1980).

Passive dispersal parameters: σ_P, δ and F. No quantitative data exist on passive dispersal by soil transported on agricultural machinery. A first survey revealed that soil transported by tractor wheels contained worms in all life stages. To mimic transport of cocoons by soil on tractor wheels, coloured beads of cocoon size were introduced into the soil and dispersed by a tractor under wet conditions. The number of beads in the track over 65 was assessed. More details about the method are given in Marinissen (1991).

δ is the probability that a cocoon will survive passive transport. In the absence of experimental values for δ the model was run with a range of values. The chance for an individual to be transported, F, is estimated from (a) the percentage of the field affected by tractor wheels, b) the estimated percentage of cocoons deposited yearly in the 0-5 cm layer of the soil and c) the percentage of beads removed by tractor wheels. Between 36 and 51% of the glass beads were taken up from the inoculation plots during the experiment.

In Table 2.2.1A and 2.2.1B the parameters we used for different species in arable land are listed. For some unknown parameters a range of values was used in the calculations.

Table 2.2.1A Estimated parameter values and predicted velocities of population expansion in arable land (using Eq. 6) for *Aporrectodea caliginosa* and *Lumbricus rubellus*

species	cocoons[1)]	R_0[2)]	σ_A[3)]	σ_P[4)]	F[5)]	δ	C[6)]
L. rubellus	90	15.8	2.4	10.5	0.068	0.01	11.35
						1.0	11.51
A. caliginosa	30	5.3	1.0	10.5	0.043	0.01	5.70
						1.0	5.77
			2.0			0.01	6.98
						1.0	7.10

Table 2.2.1B As in table 2.2.1A for the species *Aporrectodea rosea* and *Lumbricus terrestris*

species	cocoons[1)]	R_0[7)]	σ_A[8)]	σ_P[4)]	F[5)]	δ	C[6)]
A. rosea	8	1.4	0.25	10.5	0.026	0.01	1.75
						1.0	1.82
		3.0				0.01	3.27
						1.0	3.31
L. terrestris	5	1.0	0.25	10.5	0.0017	0.1-	0.00
			10.0			1.0	0.00
		1.1	0.25				0.28
			1.00				0.62
			10.0				5.68
		2.0	0.25				0.77
			1.0				1.67
			10.0				15.31

1) Number of cocoons per adult per year (Evans and Guild 1948)
2) Offspring per adult per year, calculated as cocoons(1) x fraction hatching x fraction of juveniles surviving until adulthood. See Methods for further details
3) Measured for *L. rubellus* using pitfall traps (see Methods). For *A. caliginosa* we used two estimates, both lower than 2.4, because we expected that the active dispersal would be lower than for *L. rubellus*
4) Measured in the glass-bead experiment
5) Fraction of cocoons being transported, calculated as fraction of cocoons taken up (from glass bead experiment =0.5) x fraction of surface under tracks (=0.2) x fraction of cocoons in upper 5 cm of soil
6) In m year^{-1}
7) Offspring per adult per year, calculated as cocoons(1) x fraction hatching x fraction of juveniles surviving until adulthood. Because we had no estimates of hatching success and survival rates for these two species we varied the value of R_0
8) Expected was that *A. rosea* has lower active dispersal than the species of table 2.2.1B. For *L. terrestris* we took a wider range of values

Validation of the model

Lovinkhoeve experimental farm was established in 1953. After seven years, earthworms reached a density of 280 per m^2 at a grassland plot near the farmhouse (Van Rhee 1963). From this grassland colonisation of the adjoining arable fields started. The distribution of earthworms over the farm was studied by Marinissen (1991). This "whole-farm" distribution, established in 35 years, was used to validate predictions of the model.

Recently started earthworm colonisation from one field to another on the farm enables a second comparison with model predictions. Since 1985, fields previously under conventional management received more organic matter (manure and crop residues) and "spontaneous" dispersal into these fields has started since. In the first year no traces of earthworm activity, such as surface casting, were detected. In autumn 1988 one of the fields was surveyed every 4 m (handsorting of 25x25x25 cm soil) over a distance of 40 m in two lines: one in a wheel track zone and one between tracks. From this survey the velocity of population expansion was calculated. In further sections of the paper we will refer to this observations as the "plot-scale study".

The model was further tested for grassland situations, assuming that σ_P is negligible in grassland. Therefore, C was calculated using Eqs. 1 and 3, and γ=0. The predictions were compared with published data.

Results

Parameter estimation

From the inoculation experiment in the arable field a σ_A of 2.4 was calculated for *L. rubellus*. For grassland σ_A was 4.1 for *L. rubellus* and 3.8 for *A. caliginosa*. No *A. longa* was found outside grassland the plots. Passive dispersal of beads in arable land yielded a σ_P of 10.8 for beads pushed in the wheel track (cocoons transported that way probably would have a low survival), and 18.3 for beads transported in clods falling off the wheel next to the wheel track. The total distribution had a σ_P of 10.5. Note that σ_A(grassland) is larger than σ_A(arable), and that σ_P is much larger than both σ_A values.

Validation of the model

Model predictions of the velocity of population expansion, C, for *L. rubellus* and *A. caliginosa* in arable land are not very sensitive to the value of δ (Table 2.2.1A). The predicted C value for *L. rubellus* is about 11 m $year^{-1}$, and for *A. caliginosa* roughly between 5.5 and 7 m $year^{-1}$.

From the whole-farm distribution of the Lovinkhoeve farm as observed by Marinissen (1991) it is seen that in 35 years *A. caliginosa* dispersed about 175 m, and *L. rubellus* dispersed 195 m. This amounts to an annual dispersal of 5 and 5.6 m $year^{-1}$, respectively, for the two species. The value for 35 years colonisation of *A. caliginosa* is within the range predicted by the model. However, the model overestimates whole-farm dispersal of *L. rubellus*.

The dispersal into the newly colonised arable field (plot-scale study) proceeded farther in wheel-tracks than between them (Table 2.2.2). For this study, model predictions are in close agreement with observed velocities of population expansion. The qualitative difference between the two species is well predicted.

Table 2.2.2 The velocity of population expansion, C (in m year^{-1}), of two earthworm species (plot-scale study). For further explanation see text

	in track	between tracks	mean
A. caliginosa	7.5	6.5	7.0
L. rubellus	15.4	10.9	14.1

The measured σ_A in grassland for *L. rubellus* and *A. caliginosa* corresponded to a velocity of population expansion of 12.8 and 9.3 m year^{-1} respectively. Values of C found in the literature for grassland range between 2.5 and more than 10 m year^{-1} for *A. caliginosa*, and are larger for *L. rubellus* (Table 2.2.3). Comparing estimated and predicted velocities for these two species in grassland show that they are in fair agreement, the calculated values tending to be slightly higher than literature data.

Table 2.2.3 The velocity of population expansion, C (in m year^{-1}), estimated from literature data. All estimates concern grassland

Management	Species	C	Reference
ungrazed orchard grass strips	(1)	6	Van Rhee 1969
grazed polder grassland	(1)	9	Hoogerkamp et al. 1983
grazed cut-over peat	(2)	>10	Curry & Boyle 1987
	(1)	2.5>x<10	" " "
pasture	(1)	8	Hamblyn & Dingwall 1945

(1) *A. caliginosa*
(2) *L. rubellus*

General patterns

Velocities of population expansion were calculated for different values of R_0, F, σ_A, σ_P and δ. For initially small values of F, increasing F led to a large increase in the rate of population expansion, C, especially when σ_P is large compared to σ_A. Furthermore, the velocity of population expansion is sensitive to σ_A, δ, and R_0, especially within the range R_0=1 to R_0=15. High values of F and high mortality during transport (low values of δ), result in reduced velocity of population expansion, especially when net-reproduction (R_0) is small (Fig. 2.2.2A, B and D). This is because the larger F becomes, the more individuals die due to the transport by the tractor wheels. When the probability of surviving transport is small, so many individuals die that the population is unable to maintain itself. Therefore C approaches 0 when F approaches 1 and δ is small.

Discussion

The velocity of population expansion predicted by the model for *A. caliginosa* is in close agreement with the velocities estimated in the field. This applies to both the arable and the grassland situation. In agreement with model predictions, *L. rubellus* colonised new fields faster than *A. caliginosa*. In examining the differences in the parameter values estimated for both species (Table 2.2.1A), it becomes apparent how differences at the individual level affect the population level. *L. rubellus* has a greater net-reproduction (R_0). This species also lives closer to the surface which results in a larger fraction of the individuals being passively dispersed (larger F). Moreover, *L. rubellus* covers larger distances during active dispersal (larger σ_A).

Predicted velocities of population expansion for *L. rubellus* are in reasonable agreement with field estimates, although the model tends to over-estimate the velocity. This is most obvious when comparing predicted velocities with the dispersal pattern over the whole Lovinkhoeve farm, where after 35 years *L. rubellus* has dispersed only about 20 m farther than *A. caliginosa*. A possible explanation for the over-estimation might be the effect of occasional adverse climatic conditions. Most parameter values are estimated under assumed optimal conditions. Bengtson et al. (1979) found that survival of newly introduced *L. rubellus* was less than that of *A. caliginosa* under the severe climatic conditions in Iceland. According to MacArthur and Wilson (1967), the probability of successful colonisation increases rapidly as the ratio of birth rate to mortality rate increases. Under adverse conditions, a decreasing death rate favours dispersal more than an increasing birth rate. *A. caliginosa*, in contrast to *L. rubellus*, can go into quiescence, which may reduce the species death rate and could thus be valuable in the colonisation process.

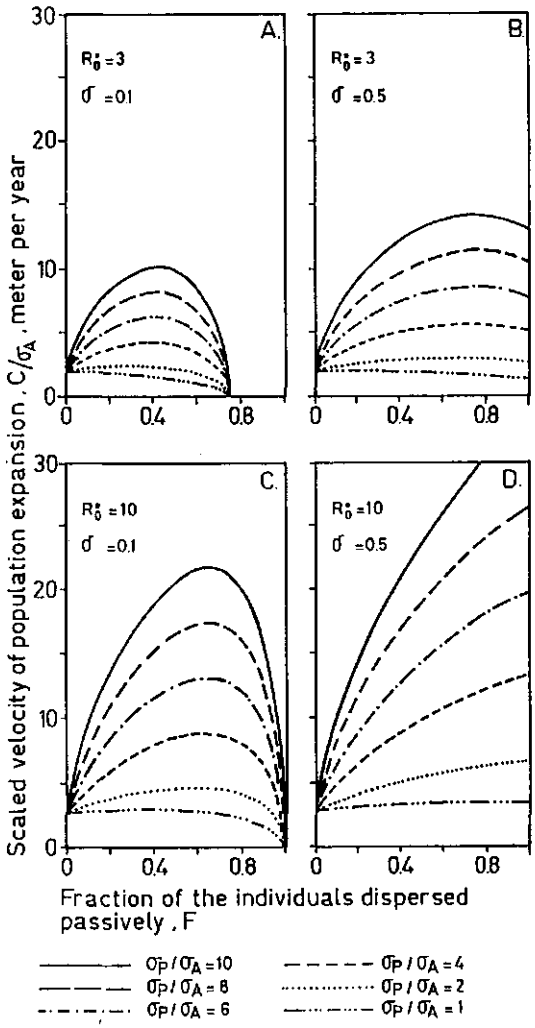

Fig 2.2.2 Scaled velocity of population expansion (indicated by C/S_A in m yr^{-1}) as a function of the fraction of the individuals transported passively (F). Lines are calculated from Eq. 6

The *C* values found for *L. rubellus* in the newly colonised field (plot-scale study) was much closer to predicted values. This might be because colonisation took place in a year with optimal conditions for dispersal: an application of manure in spring, followed by a very wet summer led to very high numbers of cocoons in the top layer of the area of the farm already inhabited by earthworms (Marinissen, 1992). Moreover, due to wet autumn conditions, much soil was transported during tillage and harvest.

Long-term model predictions may be less reliable, because conditions vary among years, which may affect R_0 and *F* in particular. Model calculations of *C* are sensitive to both these parameters. In wet years, the values for R_0 and *F* used by us may be realistic. In drier years, however, both production of cocoons and transport of soil is much less, leading to much lower values of *C*.

Similar over-estimations of dispersal rates can be expected for dispersal in grasslands that developed a root mat before introduction of earthworms. Stockdill (cited in Lee 1985) followed the annual expansion of *A. caliginosa* into New Zealand pastures. After very slow expansion for 4-5 years, dispersal rates rapidly increased to up to 10 m year^{-1} (comparable to European situations). In the 4-5 year lag-phase the root-mat provided ample food to newly introduced earthworms, which may have limited their need to move in search for food (Martin 1982). After total consumption of the root-mat, the increased search for food might have enhanced active dispersal. In the model, such site-specific σ_A has not been incorporated.

For species other than *A. caliginosa* and *L. rubellus* no specific parameters are available, but tentative results can be obtained by the model, using parameter estimates as given in table 2.2.1B. For *Aporrectodea rosea*, *C* estimated this way is in the range of 1.8-3.3 m, which is in agreement with the findings of Curry and Boyle (1987) that this species does not spread farther than 2.5 m year^{-1}. A species highly valued for its improvement of the soil drainage, *L. terrestris*, is known to wander over the surface at nights, probably to find sexual partners. During these trips the worms make tracks of more than 19 m in length (Mather and Christensen 1988). However, most tracks are curved, and worms tend to keep close to the area they started from. The species has a low cocoon production, and therefore a low R_0. Its predicted spread in arable land would be 0 with R_0=1 and between 0.8 and 15 m when R_0=2. For grassland these values would be comparable, because with a very low *F* value Eq. 1 resembles Eq. 6 very closely. The calculated values for *L. terrestris* and *A. rosea* are probably overestimations, because μ=0.75 is a low estimate for these *K*-selected species.

The model might be suitable for predictions of dispersal patterns on a scale larger than individual fields, where other means of passive dispersal also play a role. For example, Schwert and Dance (1979) showed that viable cocoons can be transported by streams. In sloping landscapes, earthworms can be washed away with eroded soil (Atlavinyté and Payarskaite 1962). Some species have eury-haline cocoons, which survive transport over sea (Frenot, in press). In the model these cases can be accommodated by assuming very small values for *F* and δ, such as 0.001, and high values for σ_P, in the order of several hundred meters to a few kilometres. In this case σ_P/σ_A is between 100 and 1000. With an R_0 between 10 and 30 and σ_A=2 this leads to velocities between 20 and 220 m year^{-1}. This is well in the range described by Lee (1985). He compared observations of Benham (1905 and 1915) and Lee (1953) on earthworm populations of the Island of Raoul (c. 3000 ha) in the southwestern Pacific. Benham only occasionally recorded earthworm populations in

1905, including a few *A. caliginosa*. Lee (1953) found in 1949 that *A. caliginosa* was widespread and numerous in A-horizons of forest soils throughout the island. Assuming a circular shape of the island and the starting point at the centre, the dispersal rate should have been approximately 60 m $year^{-1}$. For more certain calculations, however, better quantitative knowledge on passive dispersal parameters is necessary.

The model describes colonisation as a continuous process in time and space. In reality, the border of the population will not be a smooth line. Especially when passive dispersal mechanisms play a role, expansion will proceed locally, originating from the central population like sparks from a fire. Still, to describe the dispersal in general terms and give an estimate for the order of magnitude of the dispersal, the model and its parameters perform well.

Implications of the model

The model can be used to gain insight into ways of promoting colonisation of earthworms. When, for the species under consideration, all parameter values are estimated, Eq. 6 can be used to investigate the effect of variations in parameter values on the velocity of population expansion. Management then can be directed towards influencing those parameter values that have a large positive influence on population expansion. From Fig. 2.2.2 it can be seen that the management measures to be taken depend strongly on parameter values. Increasing the probability of passive dispersal can be either positive or negative. For the two species considered in this paper, an increase in passive dispersal will have a positive influence on population expansion if by induced morality passive dispersal is not too large. It is also possible to enhance the net reproduction, R_0, of the dispersing population. Application of large quantities of nutritious food may stimulate both cocoon production and the rate of growth, resulting in a quicker maturation (smaller μ). Keeping conditions for growth of founder populations optimal by e.g. irrigation during dry spells might be of help for earthworm species not able to go into quiescence.

In this paper we have shown how a simple model can be used to study the velocity with which earthworm populations expand into new habitats. It is shown that the parameters of the model can be determined using literature data and simple, though laborious experiments. The model was validated by experimental results and literature data and performed well in field situations. We can thus conclude that Eq. 6 is open for practical applications and can help in the search for effective land management systems.

Acknowledgements

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Population dynamics of earthworms in a silt loam soil under conventional and "integrated" arable farming during two years with different weather patterns

JCY Marinissen (1994)
Soil Biology and Biochemistry 24:1647-1654

Summary

Regular field estimates of earthworm populations were made for two consecutive years in two fields of an experimental farm in the "Noord Oost Polder" in The Netherlands. The fields represent different agricultural management systems: conventional management and "integrated" management (i.e. reduced cultivation and lower inputs of inorganic fertilisers and biocides as compared with conventional management). Practically no earthworms were present in the conventionally managed field. In the "integrated" field, however, earthworms were observed on almost all occasions. Three species of earthworms were found, namely *Aporrectodea caliginosa, Lumbricus rubellus* and *Aporrectode rosea.* During the sampling period numbers ranged between 0 and 400 individuals m^2, representing a biomass of 0 to 100 g fresh biomass m^2. Tillage (rotary hoeing) reduced populations by > 50%, but under favourable food and weather conditions populations recovered within 5 months. *A. caliginosa* and *L. rubellus* showed different life strategies: the former migrated to deeper layers to survive winter cold, the latter survived frost periods as cocoons.

Potential influence of earthworms on soil structure are the protection of the soil surface to slaking by the production of stable casts, and the perforation of deeper soil layers and plough pans, opening up the subsurface soil for plant roots. However, in the integrated system used in this study, cast were buried in the soil at the time they were needed most on the surface. Perforation of deeper layers occurred mainly when populations were high, which was in autumn. Early in the season, presence of earthworms could not prevent ponding during heavy rainfall.

Introduction

Arable farming in The Netherlands is highly capital-intensive. In the last decades problems have emerged, including deterioration of soil structure from use of heavy machinery, and environmental pollution, from intensive applications of fertilisers and biocides. Therefore, research is increasingly aimed at developing ecologically sound arable systems ("integrated" management). In this context a project at an experimental farm in one of the Dutch polders has been set up to gain insight in the role of soil organisms in nutrient cycling and in the interaction between soil fauna and soil structure (Brussaard et al. 1988; Kooistra et al. 1989). Earthworms represent a significant proportion of the soil faunal biomass, and may

play an important role in both the nutrient cycling and soil structure formation.

From studies in, e.g. Germany (Ehlers 1975; Joschko et al. 1989) and Great Britain (Edwards and Lofty 1980) it has been shown that earthworms can improve drainage and facilitate root growth in minimum or no-tillage systems. In The Netherlands, most field crop rotations include potatoes and sugar beets (with heavy traffic on the field during harvest), so the soil is disturbed even in minimum tillage systems. The silt loam soil of the farm is sensitive to slaking in autumn and winter and to compaction under wet conditions. It can be questioned, whether, under these conditions earthworms can reach significantly high numbers to counteract the structural soil degradation caused by the intensive use of the soil.

Methods

Description of sites and site management

The experimental fields are located on a calcareous silt loam (calcaric fluvisol according to FAO) in the Dutch "Noord Oost" Polder. Some characteristics of the soil are given in table 3.1. A 4-year rotation (winter wheat-sugar beet-spring barley-potatoes) is practised in both the conventionally managed fields and the integrated fields. Conventional management involves high rates of fertiliser application, and tillage consisting of ploughing to a depth of 25 cm. In the integrated fields fertiliser rates are lower, and nutrients are applied partly in the form of organic manure. Since 1985, tillage has been restricted to a depth of 12-15 cm. The present and former management are described in more detail by Kooistra et al. (1989). Dates of agricultural treatments in the integrated plot during the study period are given in Table 3.2. In the conventionally managed plots tillage, sowing and harvest were done at the same dates. In September 1986 a soil fumigation with 1,3-dichloropropene was carried out only in the conventional plot. In the integrated field application of pig manure cake in March 1987 under very wet soil conditions caused very strong compaction in the wheel tracks and subsequent rotary hoeing was necessary to prepare the seedbed.

Table 3.1 Soil characteristics of the experimental fields

management:	conventional	integrated
%C (0-20 cm)	2.09	2.75
Bulk density	1.31	1.26
clay (%)	20	20
silt (%)	68	68
sand (%)	12	12
CaCO3(%)	10	10
pH(KCl)	7.5	7.5

Earthworm sampling

Earthworm populations are still in a process of colonisation of the farm, but the colonisation front had advanced beyond the experimental fields before 1985 (Marinissen

Table 3.2 Detail of agricultural treatment in the integrated field during the study period

	1986		1987	1988
Crop	Winter Wheat	Grass	Sugar Beet	Summer Barley
Sowing	01/11/85	01/09/86	24/04/87	07/04/88
Harvest	19/08/86		03/11/87	
Tillage				
Seedbed[1)]	30/10/85		24/04/87	
Mechanical weed control	14/05/86		26/05 till 02/07 4x	16/05/88
Main tillage[2)]		12/11/86	05/11/87	
Fertilisers[3)]	29/10/85			
	06/05/86			
	22/05/86			
Organic manure[4)]			21/04/87	
Biocides[5)]				19/05/88

[1)] cultivator (15cm) for Winter Wheat, rotary hoeing and rolling for Sugar Beet
[2)] ploughing (15cm)
[3)] 115 kg P2O5 per ha
44 kg N per ha
40 kg N per ha
[4)] organic manure 20 ton per ha[5)] manually spraying thistles with MCPA

1991). Two fields were selected for sampling of earthworms, one under conventional management, the other under integrated management. Before 1985, both had the same crop rotation which differed from the present, and were under conventional tillage. The integrated field had a higher organic matter content (Table 3.1) because of regular application of organic manures in the past. Sampling was done at least five times a year. Each sampling, from three randomly selected subplots of 4x4 m per field, two samples of 25x25x25 cm were dug out. Samples were divided in two layers (0-10 and 10-25 cm), and the soil was subsequently washed over a sieve (0.2 mm). In this way, all earthworms, including small juveniles and cocoons, could be recovered and counted. On three occasions handsorting was used. To correct for the inefficiency of this method, the numbers of small juveniles (<200mg) were doubled (Lee 1985). All individuals were identified at species level, and classified as either small juveniles, large juveniles (>200 mg) or adults. Biomass, including gut contents, was measured on individual live worms, after keeping them in water for at least two hours. Before weighing, the worms were blotted dry with paper tissues. From April 1986 till March 1987 only total weight of cocoons was measured. From March 1987 cocoons were determined to species, and total weight was measured per species. In February 1988 an integrated field adjacent to the integrated managed field under study, with the same history as the conventional field, was sampled. In this field, sampling was done

in a linear pattern, with 5 m intervals up to a distance of 25 m from the border of the organic rich integrated field.

Statistical analysis

Population densities of the different species on different sampling occasions were compared pair-wise using the Mann-Whitney test. Species composition over time was analysed further by means of CANOCO, a canonical ordination programme designed by Ter Braak (1988, based on methods described in Jongman et al. 1987), which involves a multivariate gradient analysis to correlate species composition with external explanatory variables. In this way, the number of earthworms divided into species, development stage and sampling layer were correlated with the variables "date of sampling" (number of days from the start of the sampling period), "month", and total "rain" and mean "temperature" in the 4 weeks preceding the sampling.

Results and discussion

Abiotic data

Fig. 2.1 gives information about weather conditions. Some winters had severe frost periods, sometimes very late in winter (february 1986), but also a very wet, mild winter occurred. Rainfall in the field season of 1985 was close to average, 1986 was rather dry and 1987 was wet.

Influence of management on earthworm populations

The only specimens found in the conventionally managed field were one juvenile *A. caliginosa* in June 1986, and two cocoons, one of *A. caliginosa* and one of *L. rubellus* in June 1987. On other sampling dates, occasionally cocoon remains were found. By means of dispersal by transported soil on tractor wheels and active dispersal, described for the same fields by Marinissen (1991), the number of earthworms entering the conventional fields and the integrated fields are probably similar. The results of the linear sampling of the integrated field with conventional past management

Table 3.3 Worms populations at the integrated field with the conventional past as a function of distance to the organic rich field

Distance from organic field (m)	*L. rubellus*		*A. caliginosa*	
	Juveniles	Cocoons	Juveniles	Cocoons
	(numbers per m^2)			
5	1	11	0	1
10	0	0	1	0
15	0	0	0	0
20	1	0	0	0
25	0	0	0	0

(Table 3.3) confirms this hypothesis. In this field, no adults were found, and only a few juveniles. All cocoons were found close to the border of the integrated organic rich field, suggesting that they have been conveyed by means of tractor wheels. In the conventionally managed field, micro-morphological analysis showed some remains of earthworms channels in the sub-plough layer, where they can persist for a long time (Kooistra et al. 1989). Most probably the lack of organic material and the use of pesticides have prevented population development of any founder populations in the conventional fields. Fumigation with the nematicide 1,3 dichloro-propene is particularly lethal to earthworms (Edwards & Lofty 1978). According to El-Duweini and Ghabbour (1965) food is a very important factor determining the abundance of earthworms, so also the lack of organic inputs may play a role in the absence of earthworms in the conventional field.

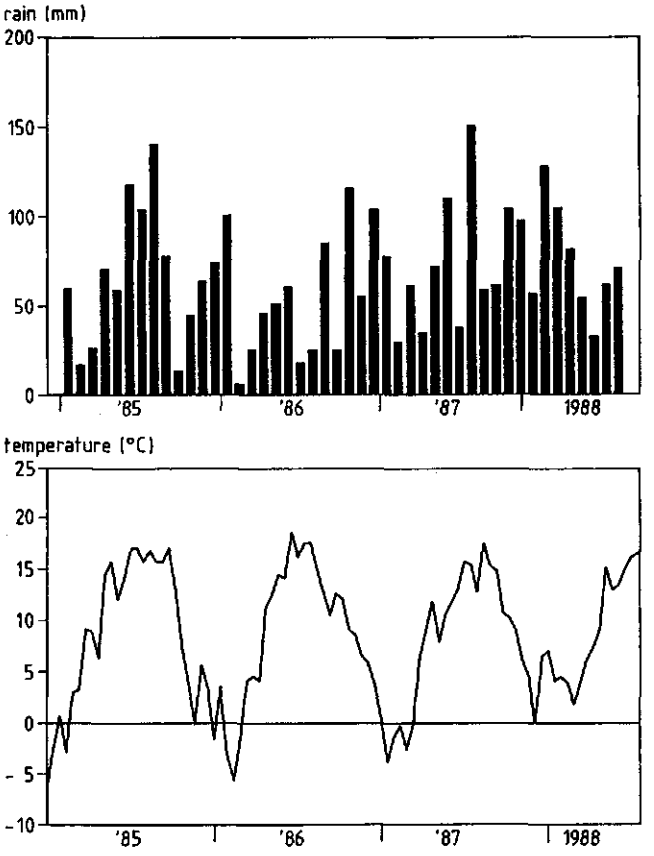

Fig. 3.1 Two-weeks totals of rainfall (in mm) and mean air temperatures (in °C) at Lovinkhoeve experimental farm

In the integrated field, population densities of up to 400 individuals per m^2 were found, representing a live biomass between 0 and 100 g per m^2. This is slightly higher than numbers given by Boström (1988) for Swedish arable land, perhaps on account of milder winters and longer summers in The Netherlands. The numbers and biomass are comparable to or slightly higher than in other studies as reported by Lee (1985) and Edwards and Lofty (1977), which may be due to the high efficiency of the method used in this study.

Species composition and population dynamics

A. caliginosa was the dominant species, both in number and biomass. The second species was *L. rubellus* (Fig. 3.2A-C). Occasionally some *A. rosea* were observed. Numbers of *A. caliginosa* (Fig. 3.2A), varied seasonally. In each sampling year, *A. caliginosa* was found as early as April. In 1987, just after the frost in February, only cocoons were present in the

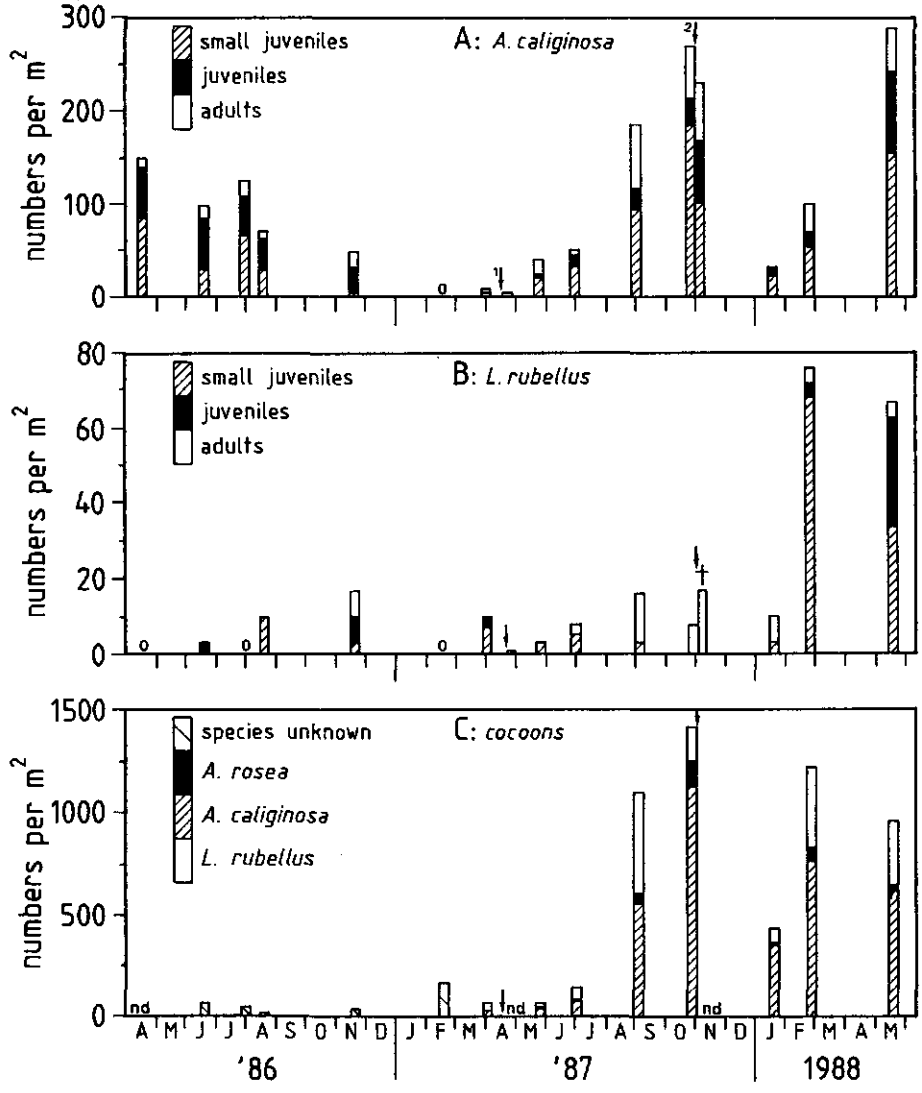

Fig. 3.2 Population densities (numbers per m^2) throughout the study period at the integrated field
n.d.= not determined, arrows: 1= application of manure cake and rotary hoeing, 2= harvest of sugar beets)

top 25 cm of soil, whereas in 1988 worms were found as early as January. In April 1986 and 1987 and January 1988 juveniles appeared first, adults later on. Mean weight of small juveniles at these occasions (Fig. 3.3A) was higher than later in the year. Given the low temperatures and a hatching weight of about 10 mg, this implies that they cannot have hatched after winter. The depth distribution early in the year differed from that in autumn (Fig. 3.4A): a significantly lower percentage of *A. caliginosa* was found in the top 10 cm in April 1987 and February and March 1988 than at the other sampling dates. In the

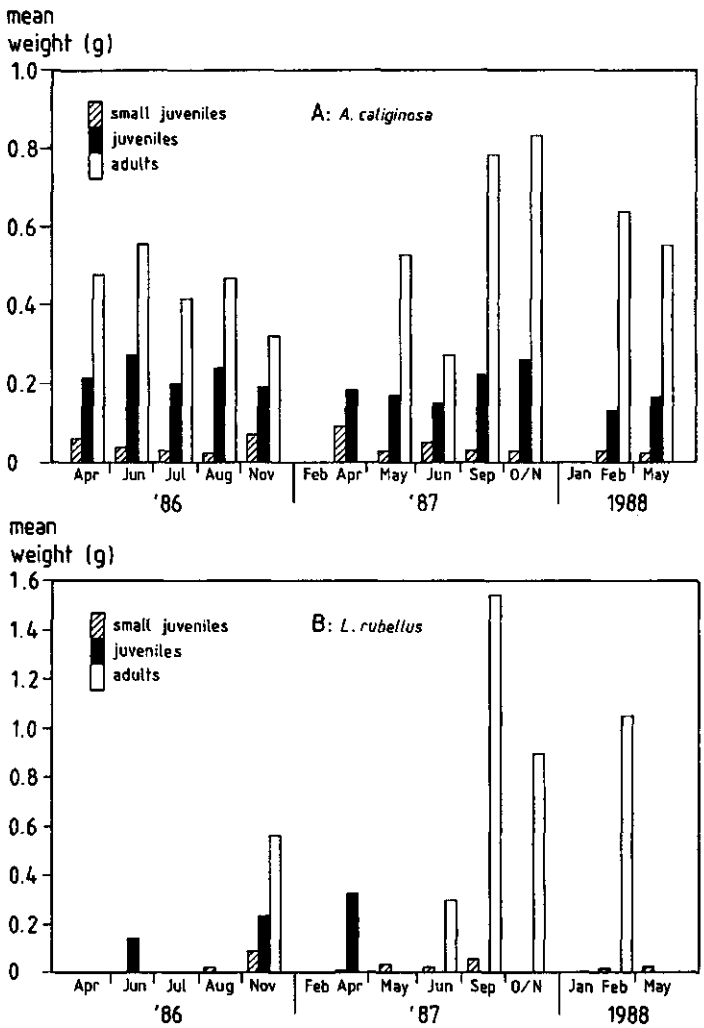

Fig. 3.3 Mean weight of individuals in different life stages (wet weight, including gut contents)during the study period

relatively dry year 1986, about half of the *A. caliginosa* population lived in the upper 10 cm of the soil against >85% during the wet year 1987. In January 1988 the population was confined mainly to the 10-25 cm layer (Fig. 3.4A).

L. rubellus mostly stayed in the top 10 cm of the soil profile (Fig. 3.4B). otherwise the relatively low numbers make it difficult to detect a clear pattern for *L. rubellus* (Fig. 3.2B). In spring 1986 and 1987 no adults were found. Adults appeared in autumn 1986 and earlier in the season in 1987, and remained present throughout winter 1987-1988. All adults found in November were dead, probably as a result of harvesting sugar beets with heavy machinery under wet conditions. The low mean weight of small juveniles (Fig. 3.3B) in April 1987 and February 1988 suggests hatching at these periods.

Adults of both species had low mean weights in November 1986 and June 1987 which might indicate that these were newly developed adults. Development into adults apparently took much more time in the dry year 1986 than in 1987. The summer of 1987 was favourable for earthworms because of its wet character and because of the organic manuring that took place. Weights of individual adult

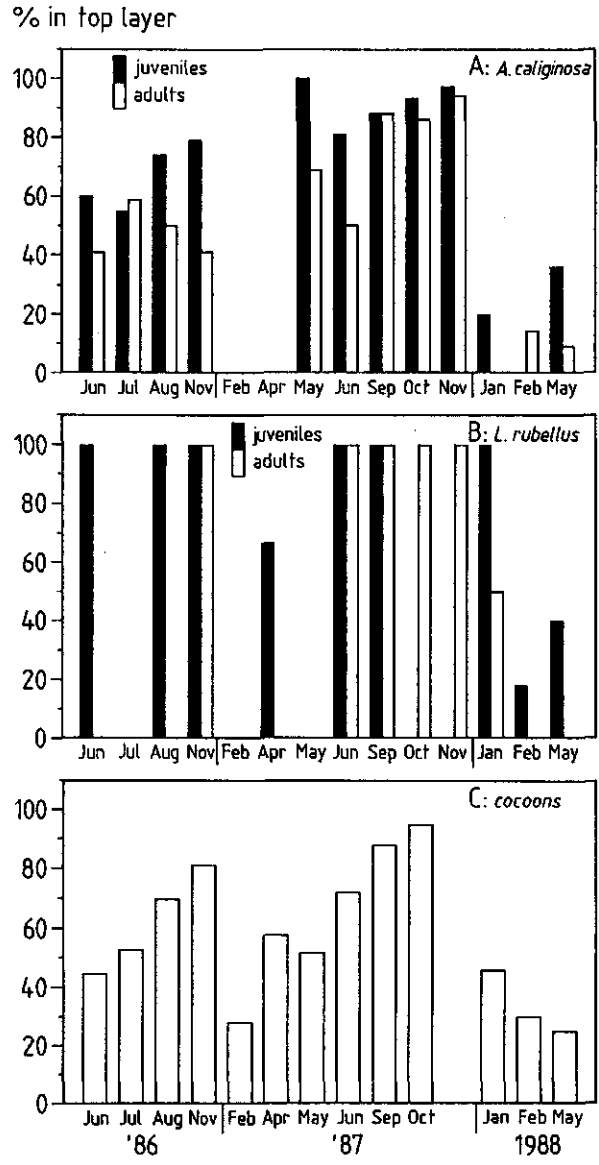

Fig. 3.4 Fraction of the total population of each species found in the top 10 cm of the soil profile

worms of both species were high. Decreasing quantities of manure cake could be found in the soil from April until September. In September *L. rubellus* had maximum mean adult weight, and highest cocoon:adult ratios. *A. caliginosa* could grow until November, when all visible traces of the manure were gone.

Cocoon numbers (Fig. 3.2c) were low in 1986 and most of 1987, but very high from early September onwards. Many cocoons survived the autumn tillage and the winter of 1987-1988. The depth distribution of cocoons parallelled that of the worms: in the dry year 1986 50% could be found in the top 10 cm, but in the wet year 1987 this increased to 70-90% (Fig. 3.4c). Gunnarson and Rundgren (1986) also found most of the cocoons in the top 15 cm of the soil. The relatively high percentage of cocoons in the 10-25 cm layer after November 1986 and 1987 may partly be due to tillage effects (ploughing to 15 cm).

The CANOCO-analysis confirmed most of the observations on population dynamics. Fig. 3.5 shows the results of the CANOCO-analysis for the first two axis in the analysis of the abundance data. The two axis together explain 92% of the variance. In the diagram, projection of the head of an arrow on an axis indicates the relative importance of a factor for that axis, and projection of the species points on an arrow indicates the response of the earthworms to that factor. E.g., the arrow for "month" runs south-east. Thus, for a particular soil layer, a species occurs most frequently early in the year if its symbol lies in the north-west part of the diagram, and later in the year if it lies more to the south-east. The factor "month" was the most important, followed by "date of sampling", "rain" and "temperature". The appearance of adults is

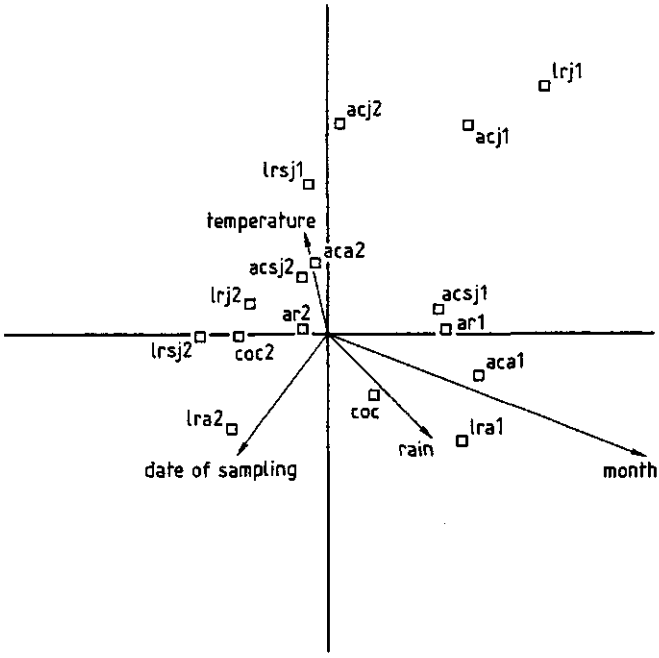

Fig. 3.5 CANOCO-diagram (ac = *A. caliginosa*, lr = *L. rubellus*, ar = *A. rosea*, coc = cocoons, j = juveniles > 200 mg, sj = juveniles < 200 mg, a = adults, 1 = individuals in top 10 cm layer, 2 = individuals in 10-25 cm layer)

linked to the factors "month" and "rain" in both *A. caliginosa* and *L. rubellus*: juveniles are located predominantly to the north-west of the adults, which is the same direction as the "month" factor and the "rain" factor. For *A. caliginosa*, the individuals in the deeper layers are located also more in the north-west part, as compared to the ones in the 0-10 cm layer. This also suggests an influence of "rain" and "month". For *L. rubellus*, the 0-10 cm inhabitants are more north-east in the diagram and those from deeper layers south-west. The factor "date of sampling" points in the same direction. This is because only in winter 1987/1988 more *L. rubellus* were found in deeper layers (Fig. 3.4B).

The factors "temperature" and "rain" lie in opposite directions, implying that higher temperatures are correlated with low rainfall, and it cannot be assessed which factor influences the earthworms most.

Life strategies of different species

The data suggest different life strategies of the two main species to survive winter periods. *A. caliginosa* survives in deeper soil layers, the adults probably even deeper than juveniles, and ascends to the surface layers in spring. This seasonal pattern of emergence of *A. caliginosa* was also observed by Boström (1988) in arable land and by Rundgren (1977) in other habitats. In this study *L. rubellus* is more strictly confined to the top soil layers. During severe frost mortality of *L. rubellus* in the frozen top layers of the soil probably will be high. Population then develops from hatching cocoons in spring, which mature to adults in autumn. During very mild winters (1987/1988) mortality is low, and individuals and cocoons surviving harvest and tillage can quickly build up high populations again if food and weather conditions are favourable.

Influence of tillage on earthworm populations

Rotary hoeing, which causes severe "non-inversion" disturbance of the soil directly killed 50% of the earthworm population (Fig. 3.2). Similar observations were made by McLennan and Pottinger (1976). Rushton (1988), however, mainly observed a more long term deleterious effect of tillage, in particular on anecic species. Edwards and Lofty (1982) even found a stimulating long term effect of tillage on some species. These different results may be due to differences in tillage and in soil type (Haukka 1988). Although in the present study the direct damage was high, the effects were not very strong. In the first place, populations were low before tillage, and the combination of manure application and a wet summer resulted in maximum densities in autumn 1987. Harvest of sugar beets caused a high mortality in *L. rubellus* adults. Under wet soil conditions the vibrations of the machinery are conducted well, causing the worms to appear on the surface, where they are crushed under the wheels. However, populations could build up in spring 1988 again after a mild winter and because of high numbers of cocoons in the soil, probably helped by a

residual effect of the organic manuring. According to Lofs-Holmin (1983), such an influence can persist for up to 3 years.

Influence of earthworms on soil structure

In autumn 1987, earthworm populations in the 0-10cm layer were high, and at least the top 5 cm of the soil consisted of earthworm casts. Using the field data on earthworm biomass, combined with Boström's data (1988) for amounts of cast production in barley crops, it can be calculated that during the autumn 1987 the bulk density of the top 10 cm soil could be reduced from 1.3 (Table 3.1) to 1.26 g/cm^3 in two months of earthworm activity. In April 1987 the application of the manure caused strong compaction in the wheel tracks. At tillage in November, a grey-coloured anaerobic layer was observed in the tracks at *ca* 15 cm depth. This anaerobic layer was perforated at regular intervals by aerated (brown colour) earthworm channels. Just before tillage, air permeability in wheel tracks was much higher in the presence of worms than in their absence (Marinissen unpubl. results). However, early in the field season, heavy rain caused formation of pools on the surface even in earthworm plots, probably because populations of earthworms were not yet strongly developed and/or active. The Lovinkhoeve soil is very sensitive to slaking, and continuous pores including worm channels can get clogged easily by infillings (Kooistra et al. 1989). In autumn 1987 the top 5 cm of the soil totally consisted of earthworm casts. Fresh casts are more susceptible to dispersion than uningested soil, but become more stable when dried (Marinissen and Dexter 1990). Also, when worms are fed fresh organic materials, stability of dried casts is enhanced even further (Shipitalo and Protz 1988). In September 1987, 75% of the casts sampled from the integrated field were stable (wet sieving) as compared to 20 % for uningested soil aggregates from the same plot (Marinissen, 1994). So, the layer of casts could protect the surface from slaking. However, during harvest of potatoes or sugar beets, or during autumn tillage uningested soil will be brought to the surface again. Therefore, under these circumstances, short-term positive effects of worm casts on the soil stability against slaking cannot be expected.

Worms can play a role in maintaining a favourable soil structure if food and weather conditions are favourable. However, it takes some time for populations to develop, especially in climates with severe winters. The winter migration of *A. caliginosa* leads to perforation of deeper soil layers, including any plough pans, that are not normally reached by tillage. In this way, pores are created for rooting in layers under the tilled zone. *Lumbricus terrestris* and *Aporrectodea longa* are even more efficient deep burrowers, but do not survive heavy soil disturbance (Edwards and Lofty 1982). So, only in systems without turning of soil and burial of surface casts can earthworms help increase soil structural stability.

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Earthworm populations and stability of soil structure in a silt loam soil of a recently reclaimed polder in the netherlands

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Abstract

Earthworms are believed to be beneficial to the quality of the soil structure. An important aspect of quality of soil structure is the stability of aggregates. The influence of earthworms on soil structure stability was investigated in a silt loam soil under "conventional" (high-input) and "integrated" (reduced-input) management. Aggregates from the top 5cm of soil were sampled, air dried and analysed for their size distribution and water stability. Dry-sieved aggregate size distribution did not differ appreciably among fields and between years, so aggregates of a certain size represented a similar fraction of the soil for all fields. The wet-sieving procedure, used to measure aggregate stability, yielded highly reproducible results. Within-field variability of the stability was lower than between-field variability, so in further sampling only one mixed sample per field was analysed for soil structure stability. Analysing only a large and a small aggregate fraction was enough to evaluate aggregate stability in this study, as there was a gradual increase in stability from large to smaller aggregates. A 2 year time series of samples was analysed for earthworm population density and aggregate stability. Aggregate stability showed a seasonal pattern and was correlated significantly with earthworm density. Other factors influencing aggregate stability were management history in the conventional and integrated fields and, for a wider range of fields, organic matter content of the soil. The Lower Plastic Limit, another stability parameter, was influenced mainly by organic matter and clay content, and not by any of the seasonally changing factors.

Introduction

In reviews of the influence of earthworms on ecosystems, numerous beneficial effects of earthworms on soils are listed. However, their importance to arable systems is not always clear. In this study the influence of earthworms on soil structure stability is evaluated, mainly by comparison of two management systems: "integrated" (INT) versus "conventional" (CONV). The integrated system applied here involved lower inputs of fertiliser, increased organic inputs and reduced application of biocides compared with the conventional system. Tillage depth was also reduced to avoid some of the disturbance of the soil fauna. In the integrated system biological processes were expected to be important for nutrient cycling and disease control, and maintenance of soil structure. In this paper the

influence of earthworms on the quality of the soil structure, as expressed by aggregate stability, is tested.

The experimental set-up involved fields that had been divided in two halves (A and B) since 1953. Block A repeatedly received organic manures during an 8 year rotation including 2 years ley, whereas block B only received inorganic fertilisers during a 6 year rotation (without ley). Since 1985, the integrated and conventional blocks were laid out perpendicular to the old blocks. In this way both systems had fields with a history of organic manuring and leys and fields with a history of inorganic fertilisers only (Fig. 2 in Lebbink et al. 1994). In 1987 a neighbouring block was added to the experiment, on which integrated management was applied, but with minimum tillage (MTnew). Details on present and former management can be found in Brussaard et al. (1988), Kooistra et al. (1989) and Lebbink et al. (1994). At the experimental farm also other experimental plots were available, such as a long-term organic versus inorganic fertilisation plot, and a crop rotation experiment. A single sampling from these experiments provided the opportunity to evaluate the influence of single agricultural measures on earthworm populations, which helped to interpret the results of the integrated agriculture experiment of which the set-up was multi-factorial.

To evaluate the role of earthworms in the agro-ecosystems, their population densities and seasonal and yearly variations were monitored and soil structural stability was measured. Earthworms do not only influence soil aggregates by the passage of soil through their guts, they also form channels. In this way the morphology and the physical properties of the soil can both be influenced by earthworms. Information on the latter aspects of soil structure of the experimental fields can be found in Boersma and Kooistra (1994) and De Vos et al. (1994).

Methods

Earthworm counting

Earthworm populations were sampled by taking six soil samples of 20x20 cm and 25 cm deep, from layers 0-10 and 10-25 cm per sampling occasion per field. Earthworms were collected from the samples mostly by washing the soil over a sieve (0.3 mm mesh) and a few times by hand-sorting. Earthworms were counted and individual live fresh weight was recorded.

Water stability measurements

Mixed soil samples from the top 5 cm were air-dried in the laboratory, and dry-sieved into the fractions >8, 4.8-8, 3-4.8, 2-3, 1-2, 0.3-1 and <0.3 mm. Water-stable aggregates

were quantified by a modified version of the wet sieving procedure of Yoder (1936). A portion of the dry aggregates was put on a matching sieve of a sieve set, quickly immersed in demineralised water, and sieved with a stroke length of 15 cm and a stroke period of 7.5 s for 5 minutes. All fractions were collected separately, dried and weighed. The fraction of stable aggregates (percentage of total dry weight retained on the upper sieve) and the Mean Weight Diameter (MWD) of the disintegrated fractions of the aggregates was then calculated.

For some aggregates also the Lower Plastic Limit (LPL) was measured according to Atterberg (1910). This is the water content at which the soil changes from plastic deformation to brittle fraction, while drying out. The higher the LPL, the larger the chance that the soil is workable over a longer time-span.

Selection of aggregate size fractions for analysis

To test whether all dry fractions should be analysed for their stability, samples were taken from five fields in spring 1987: CONVB, MTnew, INTA, INTB and a former grassland (fG). Of all these fields the stability of the fractions 4.8-8, 1-2 and 0.3-1 mm was measured in triplicate. The results were tested with analysis of variance (ANOVA). In the former grassland the fractions 2-3 mm and 3-4.8 mm were also analysed. The stability of the five different fractions from this field was compared using Tukey's test.

Check on the variability within fields

To test wether variability within fields is so high that it necessitates replicate sampling per field three samples were taken from each of five different fields in the spring of 1988 (CONVA, CONVB, MTnew, INTA and a grassland (G) in the neighbourhood of the farm). The stability of the fractions 4.8-8 and 0.3-1 mm was measured in four replicates for each sample. A nested ANOVA was performed to test how much of the variability could be ascribed to variation among and within fields.

Seasonal variation of earthworm populations and aggregate stability

Earthworms were sampled several times between March 1986 and 1988 on the fields INTA and CONVB. The results of this sampling are reported in Marinissen (1992). Earthworms were almost completely absent in CONVB. Therefore, further sampling consisted of samples taken every 6 weeks from November 1989 to November 1990 from fields planted with CONVA and INTA and also five times between December 1990 and 1991 from two other fields which had the same crop and history.

To test the relationship between earthworms and aggregate stability the same plots were also sampled for aggregate stability of the top soil (fractions 4.8-8 and 0.3-1 mm). The results of the stability measurements were analysed by ANOVA, with field as main effect

and numbers of earthworms (either expressed as numbers of worms in the top 10 cm or in the top 25 cm) and number of the month as covariables.

Other factors influencing earthworm populations and aggregate stability

To further test the hypothesis that earthworms influenced soil structural stability a larger series of fields was sampled for earthworms in September 1991. This series consisted of (I) a rotation study (two fields with continuous winter wheat, and two fields with a conventional 4 year rotation; in the latter fields sugar beets were present at the time of sampling) and (II) a manure experiment (conventional 4-year rotation, two fields with only inorganic fertiliser, and two fields with organic manure for more than 30 years; in both situations one field was cropped to winter wheat, and one field to sugar beet). In March 1992 the same fields were sampled for aggregate analysis, and aggregate stability of aggregates 0.3-1 and 3-4.8 mm and the LPL of the macro-aggregates was measured. Data on earthworm density and aggregate stability in the integrated and conventional fields, sampled at comparable times, were included in the statistical analysis. Stability measurements were analysed by Multiple Regression, using a stepwise procedure, both forward and backward, using the factors percentage of clay and organic C of these aggregates, number of worms, crop type and manure treatment. Backward multiple regression is more conservative in keeping factors in the equation than forward regression. Factors included in the equation derived by both ways may be considered the most important.

Results

Selection of aggregate size fractions for analysis

There was not much difference in dry-sieved aggregate size distribution among different fields: the percentage of aggregates of 3-8 mm varied over the season between 22 and 30 for the integrated fields and between 23 and 35 for the conventional fields. The MWD of dry-sieved aggregates smaller than 8 mm also differed little between the integrated and the conventional system. When differences occurred, the conventional system, especially in the B-block, often had larger aggregates (Fig. 4.1). These results indicate that each size fraction of the aggregates represents a similar part of the total soil for all fields and treatments.

The ANOVA for comparison of the stability of aggregates from five fields and three aggregate size fractions (Table 4.1) showed that both factors, field and aggregate size, significantly influenced stability. In all fields, the largest size class had the lowest stability and the smallest size class had the highest stability. The significant interaction between these factors showed that the differences among stability of the different fractions was not similar in all the fields. From the former grassland the stability of five fractions was

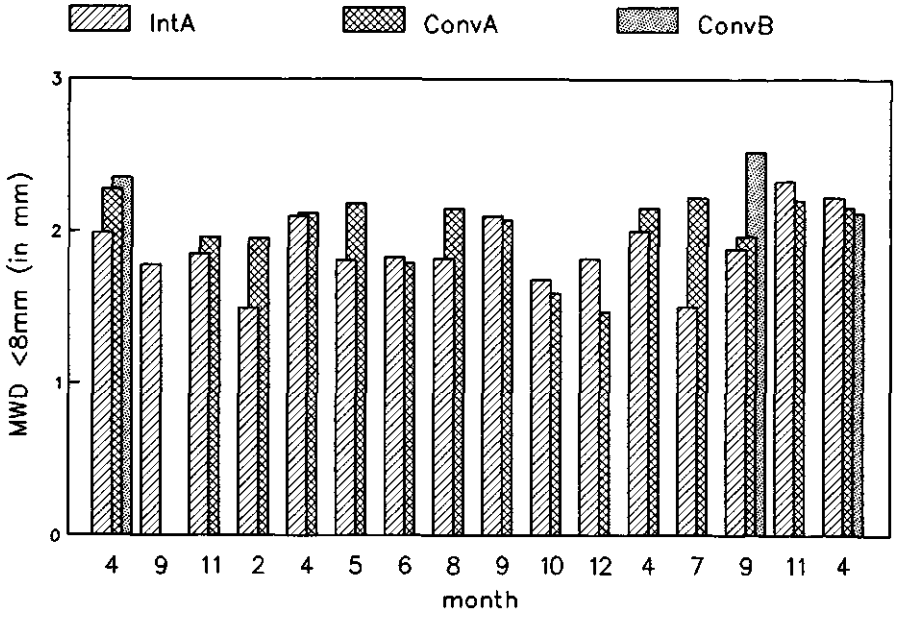

Fig. 4.1 Mean weight diameter of dry-sieved aggregates of size class smaller than 8 mm over the years

Table 4.1 Statistical analysis of water stability measurements on different aggregate size fractions from different fields.

A mean stability (%)

fraction	INTA	INTB	CONVB	MTnew	former grassland
4.8-8	19.51	18.08	6.20	29.32	39.67
1-2	52.82	48.19	19.64	41.85	77.36
0.3-1	86.70	91.04	72.16	79.61	88.80

B Anova table

Source of variation	Sum of Squares	DF	*P*
Field	7824.65	4	<0.01
Fraction	37687.86	2	<0.01
2-way interaction	2580.47	8	<0.01
Residual	339.67	45	
Total	48432.64	59	

compared with Tukey's test, which showed that the fractions 0.3-1 and 4.8-8 were both different from all other fractions, and that the intermediate size classes showed gradual differences (Table 4.2). These results indicate that considering one large fraction and the smallest fraction gives a good insight into the range of aggregate stabilities in the different fields. Therefore, later on the stability of aggregates of only two size classes was measured, herewith limiting the amount of work involved to acceptable levels.

Table 4.2 Differences between mean stability (%) of aggregates of different aggregate size classes. Columns with a different letter are significantly different at the 5% level, determined with Tukey's T-test.

fraction (mm)				
4.8-8	3-4.8	2-3	1-2	0.3-1
39.67a	67.95b	74.20bc	77.36c	88.80d

Check on the variability within fields

The results from the nested ANOVA on the data of the sampling of June 1988 (where three subplots per five fields were analysed) indicate that differences between the fields accounted for 97% of the variation for macro-aggregate stability (Table 4.3). The overall variation in stability of small aggregates was very small, and differences within fields (58% of variability explained) were even slightly larger than differences between fields (37% of variability explained). When all 9 measurements for aggregate stability of small aggregates were lumped, the differences between the fields were highly significant. Because the sieving is very laborious, one pooled sample per field was taken for the stability measurements performed later. This was analysed in triplicate or duplicate.

Table 4.3 Nested Anova for samples from different locations on five different fields

Source of Variance	Sum of Squares	DF	*P*	(Error term)	% of variation
Aggregates 4.8-8					
Field	30301	4	<0.01	location	96.84
Location	602.36	10	<0.01	error	1.91
Error	322.29	30			1.25
Total	31225		44		
Aggregates 0.3-1					
Field	600.13	4	NS	location	36.50
Location	529.07	10	<0.01	error	57.72
Error	51.19	30			5.77
Total	1180.39	44			

Seasonal variation of earthworm populations and aggregate stability

Earthworms were sampled on the A-block (former organic management) in both a conventional field and an integrated field over the period November 1989-1991. During this period, the maximum density in the conventional field was 54 individuals m^2 (Fig. 4.2). In the same period in the integrated fields on the A-block the population varied between 0 to 650 individuals m^2 (Fig. 4.2). The populations consisted mainly of *Aporrectodea caliginosa*

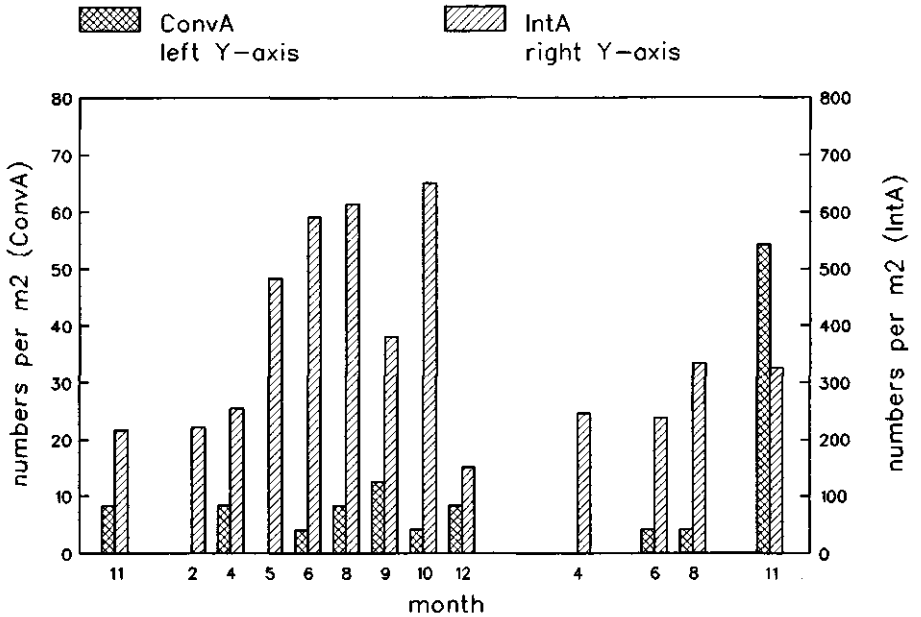

Fig. 4.2 Earthworm population densities over the season in conventional and integrated fields

and *Lumbricus rubellus*, a few *Aporrectodea rosea* were also found. As found by Marinissen (1992), more earthworms were present in spring and autumn than in winter and summer, the autumn peak being higher than the spring peak. The highest biomass was also found in autumn, coinciding with a larger proportion of adults in the population.

Aggregate stability was measured on the same occasions as the earthworm sampling in the two fields. The stability of macro-aggregates (Fig. 4.3) was very variable over the season. The highest stability was always found at the end of the field season, in both the integrated and the conventional fields. In the integrated field the stability was always significantly higher than in the conventional field. ANOVA showed that the main effect (field) was significant and the covariables (total number of worms and number of the month) also were significant. If only the number of worms from the top 10 cm was taken, there were only significant differences due to worms and month, while the field effect

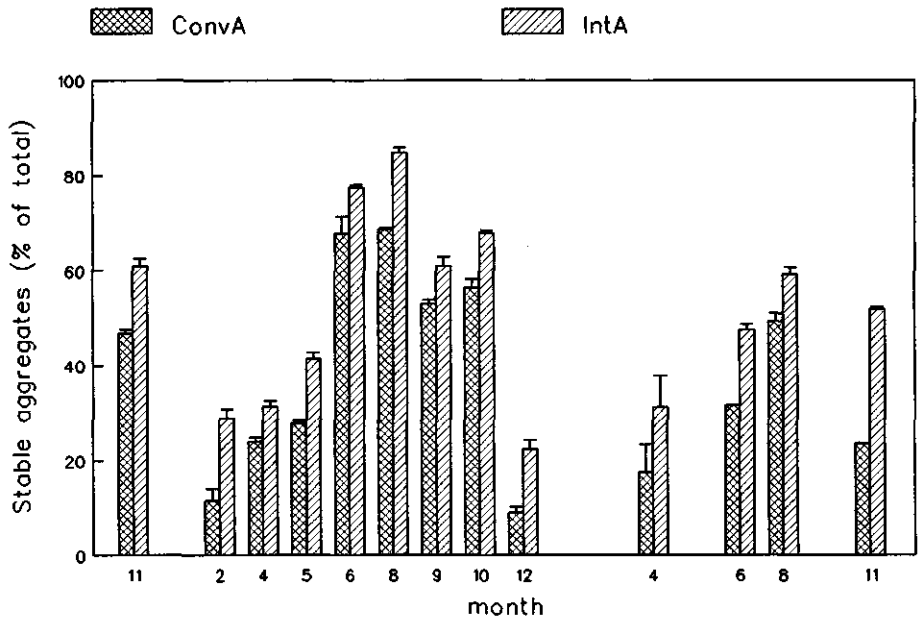

Fig. 4.3 Seasonal pattern of stability of aggregates 4.8-8 mm in the conventional and integrated fields

became insignificant. This can be explained by the fact that in the field with integrated management one-third to half of the population was always located in the deeper layers, whereas most worms found in the conventional plot were in the top 10 cm. So, differences in earthworm populations between the top layers of both fields were relatively smaller than for the whole population. The MWD of the non-stable macro-aggregates varied only slightly over the season (Fig. 4.4), and did not differ between conventional and integrated fields. This means that the degree of aggregation of smaller components into bigger aggregates was similar in both systems. Compared with macro-aggregates, the stability of aggregates 0.3-1 mm varied less with the season, and among conventional and integrated fields (Fig. 4.5). Table 4.4 lists the results of stability measurements in spring and autumn from 1987 to 1991. In spring 1988 the values for INTA and CONVA were very similar, whereas later in the experimental period aggregates from CONVA had much lower stability than those from INTA. This may be due to the fact that from 1985 onwards, the population density of earthworms started to diverge in the two fields owing to the change in management. The aggregate stability in CONVB (former inorganic management) is lower than in CONVA (former organic management).

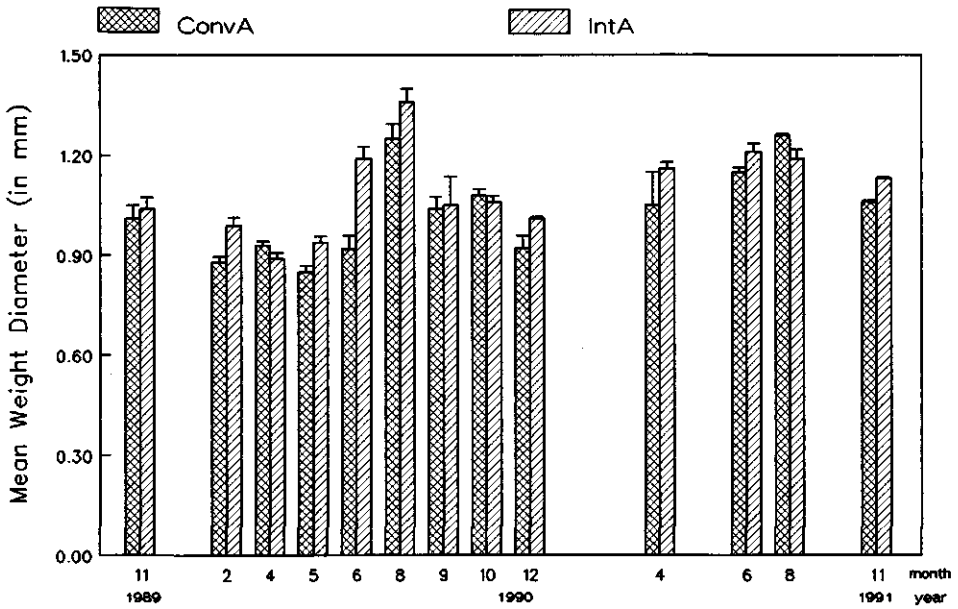

Fig. 4.4 Mean weight diameter of disintegrated aggregates after wet sieving aggregates from the size class 4.8-8 mm over the seasons in the conventional and integrated fields

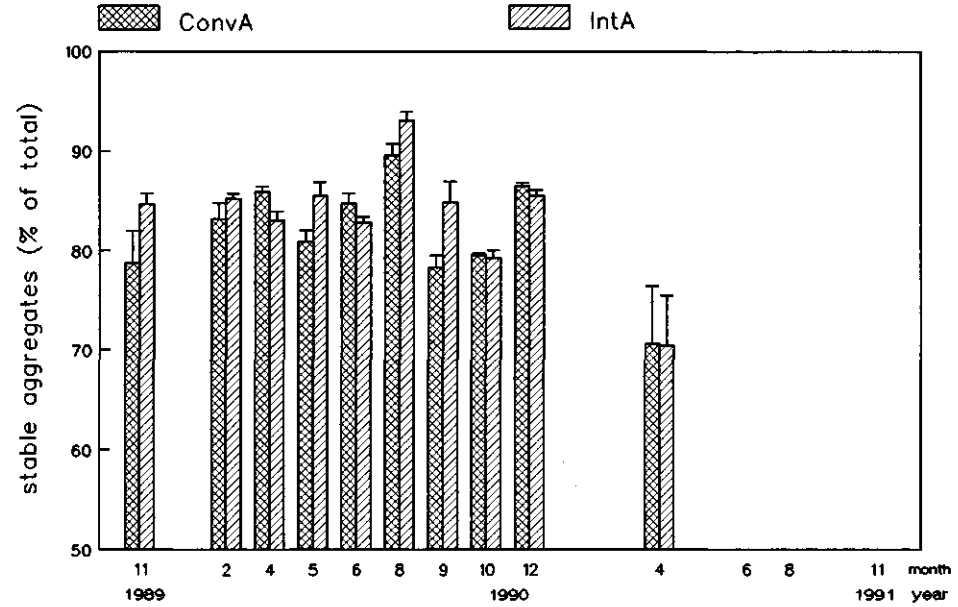

Fig. 4.5 Seasonal pattern of stability of aggregates of size class 0.3-1 mm in the conventional and integrated fields

Table 4.4 Aggregate stability (%) over the years at the Lovinkhoeve

	INTA		CONVA		CONVB		
		4.8-8 mm	0.3-1 mm	4.8-8 mm	0.3-1 mm	4.8-8 mm	0.3-1 mm
spring '87	19.51	52.82			18.08	91.04	
June '88	36.71	90.39	34.24	93.60	13.43	79.56	
Sept '88	66.59	87.54					
Nov '89	60.99	84.64	46.79	78.74			
April '90	31.35	83.10	24.00	86.00			
Sept '90	60.95	84.89	53.08	78.31			
April '91	31.22	70.42	17.63	70.06			
Sept '91	47.55		49.29		41.44		
Nov '91	51.79		23.62				

Other factors influencing earthworm populations and aggregate stability

In September 1991, in the rotation experiment, the continuous winter wheat fields had much higher populations than the fields planted with winter wheat only 1 year in 4 (Fig.4.6A). In the fertiliser experiment in the inorganic-fertilised block less earthworms were present in the field with sugar beet than in the field with winter wheat. The numbers of earthworms in the winter wheat field in the inorganic-fertilised plot were similar to those in both winter wheat and sugar beets fields in the manured block.

In April 1992, the aggregate stability of the size class 3-4.8 mm differed significantly for different fields of the Lovinkhoeve (Fig. 4.6B). The fields from the A-block and other fields, that had been under grassland previously or were under continuous winter wheat, had high macro-aggregate stability; the stability of these aggregates in the other fields was invariably lower. From the aggregates of all plots from fig. 4.6B, the content of clay and organic C was measured. Earthworms had been counted in the preceding autumn (Fig. 4.6A) in most of the plots. Backward stepwise multiple regression (excluding fG and INTB, of which no earthworm numbers were known) indicated that numbers of worms, the availability of manure and the C content each contributed significantly to the total of 73% of the explained variation. In forward regression only the number of worms contributed significantly (R^2=0.44). So earthworms had the most important effect on macro-aggregate stability, but probably manuring and C content also played a role. In both backward and forward multiple regression the variables clay content and crop type did not significantly contribute to the explained variation. The correlation between the different factors never had an *r*-value higher than 0.5, which is a requirement for the validity of the stepwise procedure.

The LPL was also measured for the different fields (Fig. 4.6C). The former grassland had the highest LPL-values, closely followed by the A-block. The winter wheat field had similar

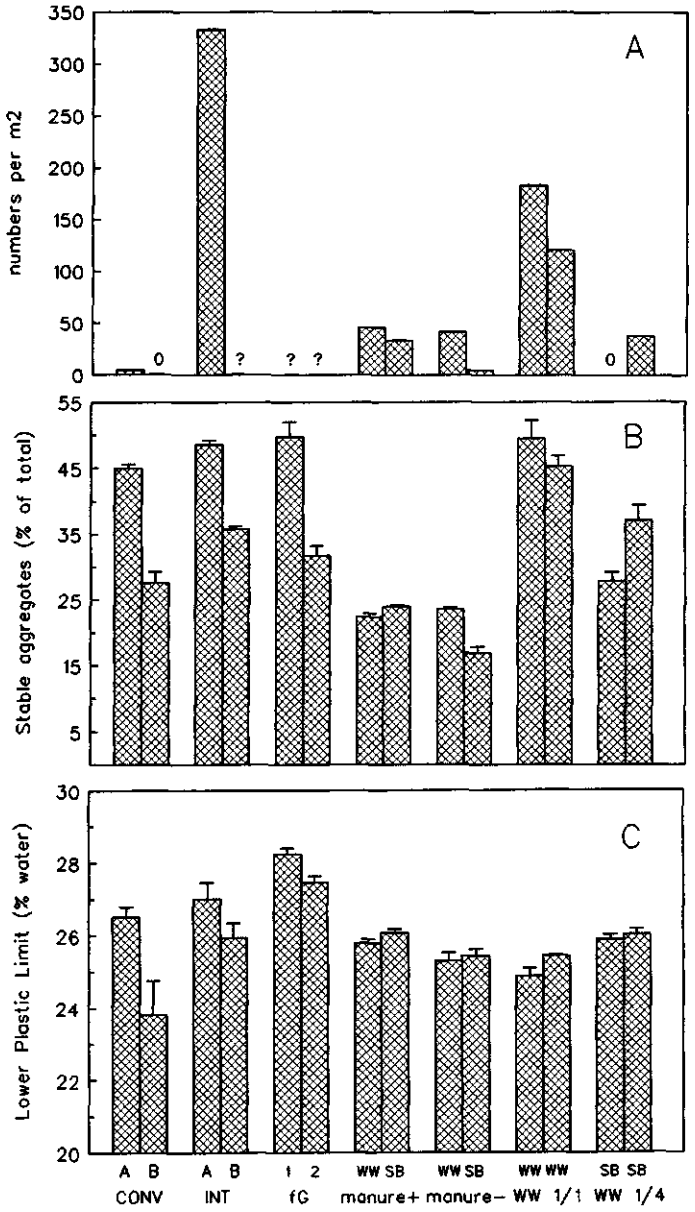

Fig. 4.6 Earthworm population density (A), stability (B) and LPL (C) of aggregates of size class 3-4.8 mm for different fields of the Lovinkhoeve, 1991/1992. CONVA conventional plot on the former A-block; INTA integrated block on the former A-block; CONVB conventional plot on the former B-block; fG 1,2 former grassland 1 (ploughed 10 years ago) and (2 ploughed 15 years ago); Manure, manure experiment with (+) and without (-) manure; WW 1/1 rotation experiment, continuous winter wheat; WW 1/4 rotation experiment 4 year rotation; WW winter wheat; SB sugar beet

values to the manure plot. Stepwise multiple regression yielded the following equation (excluding the fG and INTB):

LPL = 21.46 + 4.09*%C - 0.17*%clay (R^2=0.8)

All factors other than carbon and clay content did not contribute significantly to the explained variation.

Discussion

Earthworm populations

The maximum population density observed in the integrated field (650 earthworms m^2) is high for arable conditions. The high numbers may be related to the high efficiency of the sieving method used. Yet, although populations could reach high numbers, the development of adults will have taken several months. This slow development places the population at risk during dry summers, which hamper population growth. The relatively slow growth also makes the worm population relatively susceptible also to other hazards such as tillage operations which can reduce population by as much as 50% (Marinissen 1992).

The conventional fields were closer to the inoculation site from which earthworms dispersed over the farm, and probably were colonised earlier than the integrated fields. However, also in the integrated fields colonisation had taken place before the start of the experiment in 1985 (Marinissen and Van den Bosch 1992), but numbers may have been lower than in the fields that became conventional at that time. If we assume a similar population in the conventional fields and the integrated fields of the A-block at the start of the experiment, the data of the years 1989-1991 show that conventional management has been detrimental to earthworm populations. It is not clear how different factors of the management (e.g. tillage, pesticides, manuring and crop type) relate to this reduction. However, some indications can be derived from the measurements in the different fields. Because the species present in the field, *A. caliginosa* and *L. rubellus* are known not to be very sensitive to inversion tillage (Edwards and Lofty 1977) compared with anecic species like *Lumbricus terrestris*, the small differences in tillage depth is not likely to have been a very important factor. Fumigation against nematodes in CONV may have been very detrimental for earthworms (Lee 1985 p. 305). Marinissen (unpubl. data 1986), in a survey of plots with a range of organic matter treatments, could not find a single earthworm in the spring following autumn fumigation, although earthworms had been regularly observed in at least some of the plots by the farmer beforehand. The influence of manure can be very large (Lofs-Holmin 1983) and in the year of application and the following year there could have been a positive effect of the pig-manure, applied in INT, on the population size of the earthworms (Marinissen, 1992). However, because data on the CONVA-block were lacking

in that year, these manure effects could not be separated with certainty from weather effects. In the manure experiments elsewhere on the Lovinkhoeve farm the effect of the lack of manure was only visible in the fields with sugar beets, and not in the winter wheat fields. Winter wheat seemed to have a positive, and sugar beets a negative influence on earthworm populations, as was also indicated by the results of the rotation experiment. In the manure experiment the manure was applied before sowing the sugar beets, therefore the positive influence of the manure may have counterbalanced the negative influence of the sugar beets, while there was no such counterbalancing influence in the sugar beet field without manure. The numbers of earthworms in the manure experiment were low compared with the rotation experiment and the integrated field. In the rotation experiment the influence of winter wheat can be expected to be much higher, where this crop is grown continuously, while in the sugar beet field not only one particular field season influences the earthworm populations, but the whole 4 year rotation. This rotation was the same as in the manuring experiment. A factor which complicates the interpretation of the data of the additional experiments is that the colonisation pattern of the part of the farm where the two experiments were located is much less known than that for the conventional and integrated fields.

Only earthworm communities containing both epi-endogeics (such as *L. rubellus* and *A. caliginosa*) and anecic earthworms (such as *L. terrestris* or *Aporrectodea longa*) can influence all aspects of soil structure (Springett 1985). Population densities of the epi-endogeics were different in the integrated and conventional field, but in both systems the anecic type species were lacking. This may be a consequence of the relatively recently started colonisation of the fields. The slowly reproducing anecic species are supposed to be slow colonisers (Marinissen and Van den Bosch 1992). Another reason for their absence may be their high sensitivity for inversion tillage, because it destroys their permanent burrow system and the functioning of their "external rumen" (Lee 1985). Tillage also removes their primary food from the surface, so that they may not be able to find it. A reduction of tillage depth only, as practised in the integrated fields, is probably not enough to encourage these species to settle in arable land.

Soil structural stability

The correlation study of earthworm numbers and aggregate stability in both seasonal patterns in a few fields and in a single observation in a range of fields provided circumstantial evidence that earthworm activity influences aggregate stability. Although the aggregate stability can change quickly (within one growing season), there also appeared to be a component of aggregate stability that shows longer term temporal variation. This is indicated by the high aggregate stability of the former grassland fields, that are now under conventional treatment. Also the fields of the A-block invariably have a higher aggregate

stability than those of the B-block. This long term trend can be expected to be related to the organic matter content of the soil, which indeed played a certain role. However, the differences between C content of the fields CONVA and INTA were only small (Fig. 4 in Lebbink et al. 1994), while the difference in aggregate stability between these fields was considerable and significant. It is possible that here the earthworm activity plays a role. However, it will never be possible to distinguish between influences of organic matter and of earthworms, because fields with low organic matter inputs, and hence a low organic matter content over the years, will never harbour large earthworm populations. To estimate better the influence of the different factors, a field experiment with selective exclusion of earthworms is necessary. Preliminary results of such an experiment at Columbus, Ohio show a significant influence of earthworm population level on aggregate stability (Ketterings et al. 1993).

The influence of earthworms on aggregate stability can also be measured more directly. In a small experiment, Marinissen collected earthworm casts from the field in september 1987 and 1988 (unpubl. data). The MWD of the dried casts was 2.5 mm, while the field aggregates were generally smaller (Fig. 4.1). Casts of 4-4.8 mm were much more stable than field aggregates of the same size (Table 4.5). The difference in stability between field aggregates and casts was much smaller for the 0.3-1 mm size fraction. In laboratory experiments (Shipitalo and Protz 1988, Marinissen en Dexter 1990) fresh casts were very unstable, but their stability increased strongly with air drying. Casts placed on the surface of the soil will easily dry (and thus become stable), and may be expected to decrease slaking of the surface soil. The risk of slaking is highest in autumn. Although earthworm populations are also highest in autumn, most of the casts will be removed from the surface and mixed into the soil by autumn tillage. So, in the present system, only the presumed long-term effect of earthworms on the overall aggregate stability will influence the slaking properties of the soil. The Lovinkhoeve soil is slightly sensitive to slaking, and the slaking crust is usually more strongly developed in the B-block than the A-block (unpublished observation). Yet, the damage to the crop is not very large, because the seedlings can still break through this thin and incomplete slaking crust. Also there is a higher amount of infillings in continuous pores in CONVB than in INTA (Boersma and Kooistra 1994), but overall physical behaviour of the soil is not different (De Vos et al.

Table 4.5 Stability of worm casts and aggregates collected in the field.

	fraction (mm) 4.8-8	0.3-1
September 1987		
worm casts INTA	78.23	95.20
worm casts INTB	75.79	93.06
September 1988		
worm casts INTA	90.48	93.35
field aggregates 0-10 cm	66.59	87.54
field aggregates 10-20 cm	51.06	90.04

1994). So the clear positive influence of earthworms on aggregate stability may offer little practical advantage to the farmer in the Lovinkhoeve soil.

In this study the LPL appeared to be influenced only by organic C and clay contents, and not by earthworm activity. This may be due to the fact that the time scales of changes in earthworm populations and LPL are so different, earthworms showing high variability over one season and LPL being relatively stable over many years. This could mean that, although earthworms have disappeared as a result of recent management, such as in field CONVA, the LPL had not changed very much. Also the possible intertwining of the influence of organic matter inputs and the presence of earthworms could play a role here.

The crop rotation chosen in this study was relatively intensive, considering the amount of soil disturbance. In the case of the growth of potatoes and the harvest of sugar beets and potatoes, the soil structure is disturbed by mechanical action. Also the harvest of these crops includes high traffic loads on the fields, in the case of sugar beets at the risk of harvest taking place under wet conditions. The species-poor earthworm fauna can only play a partial role in counterbalancing these disturbances. Also the slow growth potential and the risk of combinations of hazards that may lead to low earthworm population densities imply that, although earthworms play a role in soil structure stability maintenance that cannot be replaced by tillage, in the intensive crop rotation under the relatively wet Dutch conditions, the farmer cannot be advised to omit tillage and only stimulate earthworms.

Acknowledgements

R de Fluiter is very warmly thanked for his measurements on aggregate stability and LPL and his valuable help in the field samplings. The author is also indebted to the other colleagues from the department who helped with the earthworm sampling.

Mechanisms of stabilisation of earthworm casts and artificial casts

JCY Marinissen and AR Dexter (1990)
Biology and Fertility of Soils 9:163-167

Summary

Fresh casts were collected from the earthworm species *Aporrectodea caliginosa* and artificial casts were also made. The casts were subjected to ageing, drying-rewetting and sterilisation by hexanol vapour. Clay dispersion was determined as a measure of the lack of stability of the casts. Two soils were used: the topsoil of a recently reclaimed polder soil in the Netherlands and the topsoil from a South Australian duplex-soil. In both soils fresh worm casts had higher dispersible clay than artificial casts. During ageing, both types of casts became more stable. There are strong indications that this is mainly due to changes on the surface of the casts. Fungi developed on the surface of 6-day old worm casts made of Australian soil. This resulted in a higher stability of these casts when compared with artificial casts of the same age without fungus growth. On both types of casts, hexanol inhibited fungal growth on the surface of the casts, reducing the stabilising effect of ageing. The fungus did not develop on Dutch soil casts until after 42 days, and also the development of higher stability with age was less marked than in the Australian soil. If the casts were subjected to a drying and rewetting cycle prior to analysis, they became much more stable than casts that were analysed wet. The drying-rewetting cycle removed most of the differences between worm casts and artificial casts, and also removed any effects of ageing.

Introduction

Earthworms are often regarded as highly beneficial to soils. This is because they construct channels, which facilitate penetration of air, water and roots into the soil. Also, soils rich in earthworms often have a granular, porous and stable soil structure. Much research has been aimed at measuring the stability of worm casts compared with control soil. Many authors have suggested that worm casts are more stable than natural aggregates of the same soil (p.e. Peele 1940; Swaby 1949; Brady 1984). However, more recently it has become apparent that casts are not always more stable, especially when they are fresh and wet (Shipitalo and Protz 1988). This apparent contradiction may be partly due to differences in the mechanisms of stabilisation among soil types (Emerson 1959; Dexter 1988; Dexter et al. 1988). Also, methods used to measure stability may vary highly. In particular, introducing a drying or a drying-rewetting treatment prior to analysis may strongly increase

the stability of worm casts in some experiments, as a result of irreversible changes in structure.

Several different processes may play a role in changing the stability of worm casts. If soil is ingested by worms, large amounts of watery mucus are added to it in the anterior part of the gut (Barois et al. 1993), and the soil undergoes a thorough kneading. This moulding of the soil will break bonds between soil particles, thereby reducing stability (Griffith and Jones 1965; Blake and Gilman 1970; Utomo and Dexter 1981a). This would explain the low stability of fresh, wet casts as found in the experiments of Shipitalo and Protz (1988). Futher, fresh worm casts have a very low density because they have been moulded in the worm's gut at pressures as low as 260 Pa (McKenzie and Dexter 1987). If the casts are dried after egestion, the particles are pulled steadily closer together by tension produced by water menisci and the increasing matric potential of the water (Greacen 1960; Towner 1983). These closer arrangements of the primary particles cause stronger bonding either between clay particles, or between organic and mineral particles.

However, ageing under continuously wet conditions may also increase stability (Lütjeharms 1952; Molope et al. 1987). During wet ageing both physical (thixotropic changes) and biological processes (microbial growth) can occur. Thixotropic- or age-hardening is caused by an internal rearrangement of clay and water films, causing stronger bonding between clay particles (Utomo and Dexter 1981a). Maximum thixotropic hardening occurs at water contents around the Lower Plastic Limit of the soil and becomes less significant when soil is drier or wetter than this. The particle rearrangements take place mainly during the first 10 days after moulding (Molope et al. 1987 and 1985a; Utomo and Dexter 1981a), or even, according to Arya and Blake (1972), during the first 24 hours.

Microbial growth, also, can cause increases in stability. Bacterial cells or colonies can form gel coats, to which clay particles can adhere, thus forming micro-aggregates (Foster 1978; Emerson et al. 1986). Fungi tend to bind particles directly with their hyphae (Tisdall and Oades 1982), and grow on the outside of aggregates, thus stabilising macro-aggregates. The influence of fungi reaches a peak, according to most studies, after 10-15 days (Aspiras et al. 1971; Metzger et al. 1987; Molope et al. 1987), but Griffith and Jones (1965) found a measurable influence on stability for up to 36 weeks.

From the foregoing, the following hypotheses about stability changes in earthworm casts were formulated. Fresh, wet casts are unstable after moulding in the worm gut. During ageing they will become more stable by thixotropic processes and binding through microbial activity. Microbial growth will be greater in worm casts compared to artificially moulded casts because of a higher organic matter content. Bacterial stimulation will lead to micro-aggregate formation, while fungal growth will stabilise the casts as a whole. Drying and rewetting casts will increase their stability, independently of other treatments. An experiment was designed to test these hypotheses.

Materials and methods

Soils

Two soils with a similar texture, but highly different in the degree of development, were used in the experiment. The older soil was sampled from a site near the Mount Bold Reservoir in South Australia (35°04'30''S, 138°42'50''E). The young soil came from the experimental farm "Lovinkhoeve" in the Noord Oost Polder (Land Parcel S38) in the Netherlands. Data on texture and chemical composition are given in Table 5.1. The Mount Bold soil was sampled and slightly dried before sieving with a 2 mm sieve. The polder soil was air-dried, sealed into plastic bags, packed into sealed containers, and sterilised by γ-radiation before being surface-mailed to Australia, where it was sieved through a 2 mm sieve. The fractions <2 mm of both soils were wetted to 50 cm suction on porous ceramic plates.

Table 5.1 General characteristics of the soils used. Percentages are mass fractions to oven dried soil

	Soil	
	Lovinkhoeve (The Netherlands)	Mount Bold (South Australia)
landuse	Arable	Grassland
% C	2.8	4.8
% $CaCO_3$	9.3	0.2
pH	7.3 (KCl)	4.8 (1:5 soil/H_2O)
texture:		
% < 2 μm	20	13
% 2- 50 μm	68	43
% 50-2000 μm	12	44

Earthworms

Worms (*Aporrectodea caliginosa*) were collected by hand-sorting from the botanic garden of the Waite Institute and from the Mount Bold site. The worms were kept for a week in moist soil of the same type that they would be put into during the experiment. Fifteen worms were kept per 5-litre plastic container, filled with 2 kg of wet soil (50 cm suction). The lids of the containers were lined with moist filter paper to keep air humidity high in the containers. The containers were stored in a climate room with a day/night regime of 12/12 hours, with temperatures of 16°C at daytime and 14°C at night. No food was added to the containers. There were two containers with Lovinkhoeve soil and three with Mount Bold soil.

Collection of worm casts

The worms were hand-sorted from the soil in the containers, rinsed clean from adhering soil and placed overnight on moist filter paper in individual Petri dishes. The filter paper had been wetted with de-ionised water and brought to 50 cm suction on suction plates. The

had been wetted with de-ionised water and brought to 50 cm suction on suction plates. The next morning, the worms were returned to the soil. The Petri dishes with the casts were kept cool with ice until analysis or further treatment.

Production of artificial casts

Artificial casts were made by moulding the wet soil for 2 minutes at a gravimetric water content of 100%, which was the water content of freshly deposited worm casts. The slurry was then shaped into small casts by pushing it through a syringe with a mouth opening of 1.5 mm. The artificial casts were slightly bigger than the worm casts. The water content of both types of casts was well above the lower plastic limits of these soils which would have been at gravimetric water contents of around 20%.

Further treatment

Both the worm casts and the artificial casts were analysed either fresh or after ageing in a pressure cell (12.5 cm diam, 11.5 cm high) at 50 cm water pressure for either 6 or 42 days. Half the samples were kept in a pressure cell containing a vial with 5 ml of the sterilant hexanol. Before analysis, half the casts were air-dried, rewetted the next day over a water vaporiser for 20 minutes, and then left on a porous ceramic plate at a suction of 50 cm for 4 hours. Ten casts were analysed for each combination of treatments.

Analysis

Individual casts were weighed in pre-weighed beakers, and 75 ml deionised water was added. The casts were soaked for 20 h. Next the soil-water mixture was stirred, and after 1.5 hours the dispersed clay was determined by pipetting a 5-ml aliquot at a 2-cm depth. The concentration of clay in the sample was assessed by measuring the turbidimetry in a Hach Ratio Turbidimeter and comparing this with a standard curve. The remaining soil-water mixture was then treated with an ultrasonic probe for 1 minute, while being cooled and stirred. This was expected to bring all the clay into suspension (Wace and Hignett 1988). The clay released was measured again by pipetting after 1.5 hours. The beaker with the remaining soil was then dried in an oven at 105 °C for 24 hours, and the dry mass determined after cooling in a desiccator.

Two different methods were used to stir the soil-water mixture after the 20-h soaking. One method used a paddle-stirrer, which only brought back into suspension the clay that had dispersed (seeped) out of the cast during the soaking, and the second method used a magnetic stirrer, which broke down the cast. With the paddle-stirrer, the capacity of the surface structure to prevent clay dispersion can be tested. The magnetic stirrer, in contrast, provides information about the dispersion potential of the clay inside the cast. If the clay is bound into micro-aggregates, the internal stability may be lower than if the micro-

degree of micro-aggregation.

Worm casts and artificial casts differ in their clay contents (Shipitalo and Protz 1988). Therefore, the amount of clay dispersed was related to the total amount of clay in the cast by obtaining the mass ratio between dispersed clay and total clay. This was called the dispersion index (DI).

Statistical testing

The measurements of the dispersion index were replicated ten times. However, because the casts of different ages were not kept in the same pressure-cell, the measurements cannot be seen as complete replications of the treatments. Therefore a 2x2x2x2x3 analysis of variance was carried out on the means of the dispersion index per combination of treatments, using the PC version of the Statistical Package for the Social Sciences (SPSS). To make the design more complete, 5 of the 10 replicates from age 0 were randomly assigned to either sterile or non-sterile. Because some internal stability values for the age of 42 days were missing, only the main effects and two-way interactions could be tested.

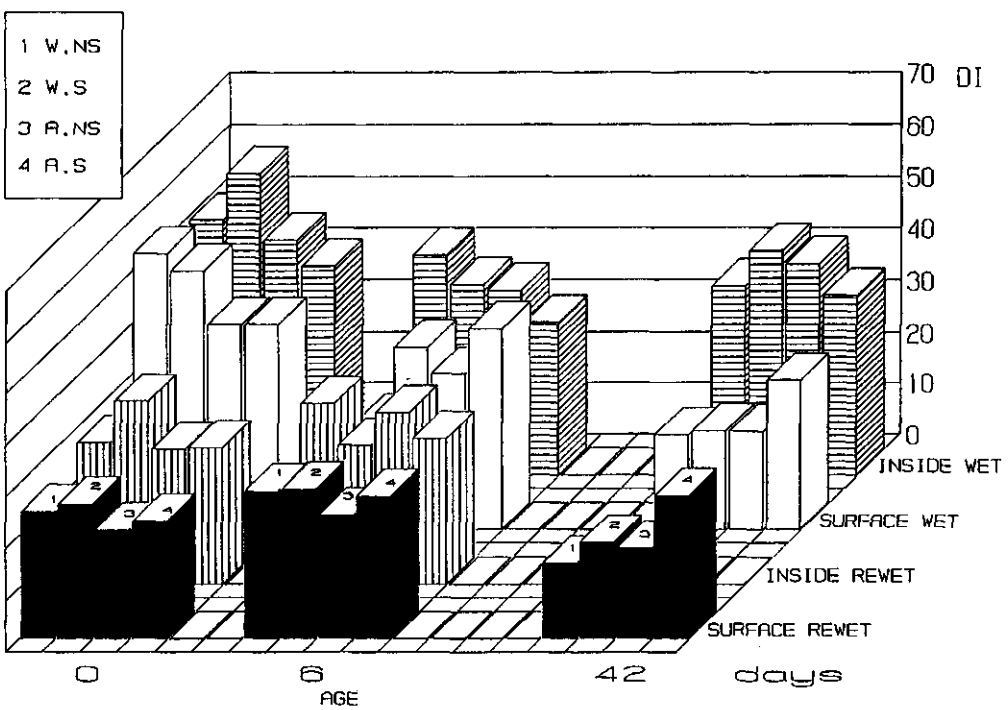

Fig. 5.1 Dispersion Index (in %) of casts made of Mount Bold soil. *W,NS* = Worm casts, non-sterile; *W,S* = Worm casts, sterile; *A,NS* = Artificial casts, non-sterile; *A,S* = Artificial casts, sterile

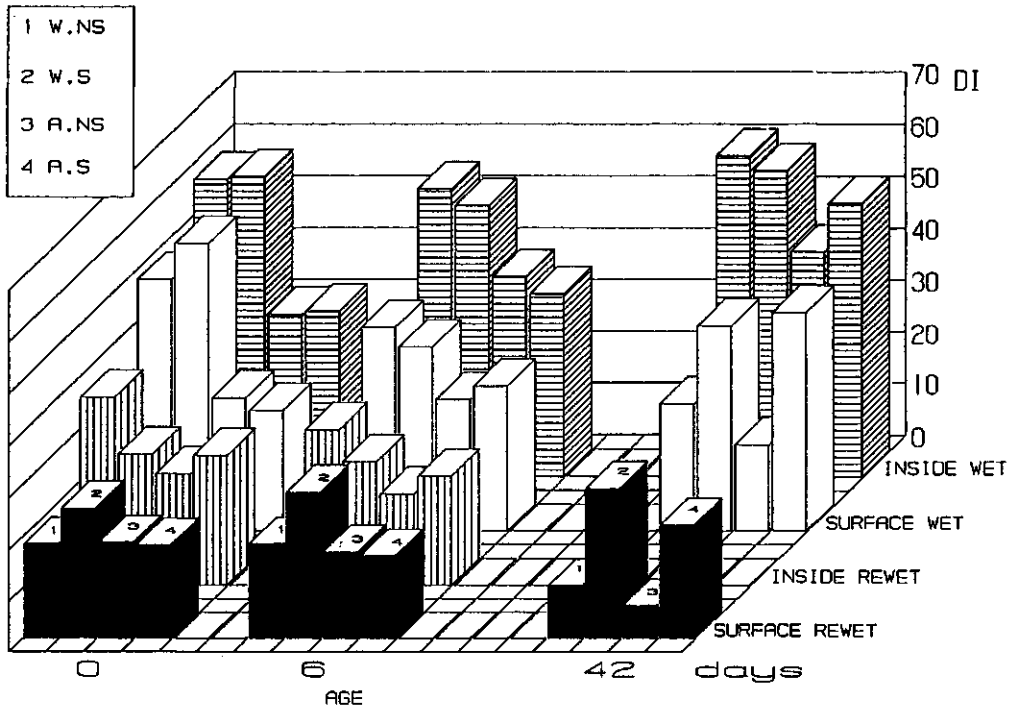

Fig. 5. 2 Dispersion Index (in %) of casts made of Lovinkhoeve soil. Abbreviations as in Fig. 1

Results and discussion

The results for the Mount Bold soil are given in Fig. 5.1, and for the Lovinkhoeve soil in Fig. 5.2. The results of the statistical analysis are given in Table 5.2. Dispersion index values ranged from 50-60% for fresh worm casts, to between 5 and 20% in dried and rewetted, aged casts. The Lovinkhoeve soil worm casts were generally less stable than artificial casts made of the same material. This was reflected in a significant main effect. Both worm casts and artificial casts become more stable with age, but most strongly in the worm casts, as evidenced by a significant interaction between the factors of age and type. After ageing for 42 days, the worm casts were still slightly less stable than the artificial casts. In the Mount Bold soil there was no significant main effect of the type factor, but there was an interaction with the age factor; the fresh worm cast were less stable than the fresh artificial casts when analysed wet, but in older casts this pattern was less clear. Differences in properties of the Mount Bold and Lovinkhoeve soils may explain the dissimilarities in behaviour of the two soils as reflected by changes in the dispersion index in the worm casts and the artificial casts. The Mount Bold soil is very old, and most of its

Table 5.2 Results of analysis of variance: *P* values

Factor	Mount Bold	Lovinkhoeve
Main effects	0.000 *	0.000 *
METHOD surface vs inside	0.000 *	0.000 *
DRY-REWET (DRew)	0.000 *	0.000 *
TYPE worm vs artificial	0.076	0.000 *
AGE	0.000 *	0.311
HEXANOL sterile vs non-sterile	0.185	0.018 *
2-way interactions	0.001 *	0.001 *
Method/DRew	0.851	0.177
Method/Type	0.157	0.450
Method/Age	0.006 *	0.016 *
Method/Hexanol	0.068	0.036 *
DRew/Type	0.364	0.002 *
DRew/Age	0.000 *	0.945
DRew/Hexanol	0.850	0.696
Type/Age	0.015 *	0.040 *
Type/Hexanol	0.548	0.521
Age/Hexanol	0.684	0.006 *

*$P<0.05$

clay is probably aggregated into micro-aggregates. The Lovinkhoeve soil, however, has undergone structural formation for only 45 years. Therefore the Lovinkhoeve soil may be more susceptible to the relatively thorough moulding inside the worm gut, than to the less intensive disruption caused by artificial moulding. This may explain why the ratio between the dispersion index of the artificial casts and that of the worm casts was often much lower for the Lovinkhoeve soil than for the Mount Bold soil.

As expected, there were also significant differences between the stability of the aggregate surface and the internal stability. In both soils the differences between these two measures of stability were smaller in fresh casts than in older ones. Throughout the experiment, the changes in internal stability in the two soils were negligible. However, the surface-related stability in both soils increased somewhat with ageing. This interaction between the factors of method and age was significant in both soils. In the Mount Bold soil there was a big difference between the wet, surface-related stability at the age of 6 days and all the other wet treatments. In the Lovinkhoeve soil the pattern was different; here the main differences were between the surface-related stability of the non-sterile treatments at the age of 42 days and all others, which was reflected in a significant interaction between the factors of age and hexanol. If it is true that the internal stability depends on the percentage of micro-

aggregates, the absence of change in internal stability during this experiment implies that practically no new micro-aggregates were formed in any of the treatments.

In both soils, a single drying-rewetting cycle led to a sharp reduction in the dispersion index and resulted in fairly constant values during the ageing. This is in accordance with the findings of Shipitalo & Protz (1988). The highest reduction in the dispersion index caused by a drying-rewetting cycle in Mount Bold soil occurred in fresh casts, and was reflected in a significant interaction between the factors of age and rewetting. In the Lovinkhoeve soil the dispersion index did not decrease significantly with age, and only a main significant effect of the rewetting factor was observed. Regardless of soil type, the worm casts and the artificial casts reacted similarly upon drying and rewetting. This suggests that organic binding materials, which are expected to differ in worm casts from those in artificial casts, do not play a major role in the enhancement of stability upon drying.

In the non-sterile samples after 6 days a net of fungal hyphae developed on the worm casts of Mount Bold soil. The appearance of the hyphae was associated with a large difference between the internal and the surface-related stability. The hyphae were still present in a melanised form on the casts after 42 days. By this time, a few hyphae were visible on the artificial casts. In the Lovinkhoeve soil, however, there was only a very slight development of fungus on some worm casts after 6 days, with more growth on both worm casts and artificial casts after 42 days. This slow rate of fungal growth compared to the Mount Bold soil may be related to the sterilisation with γ-radiation, or to other soil properties, e.g., the Lovinkhoeve soil is poor in fungal growth in field situations (JA Van Veen, pers. comm.). In both soils, hyphae were invariably absent in all hexanol treatments, but inside the casts bacterial growth occurred in all cases (Foster and Marinissen, unpublished results). The pattern of fungus growth was closely related to the pattern of surface-related stability in both soils. Because the fungal hyphae formed a net on the surface of the casts, and are known to bind particles with their hyphae, the stabilisation of the older casts from both soils was probably caused by the growth of fungus on the surface.

Conclusions

This study confirmed some of the hypotheses formulated in the Introduction. The first is that, because of moulding in the gut, earthworm casts are susceptible to dispersion when wet and fresh. This effect seems to be stronger in soils with a low structural organisation. Bacterial growth occurred in both artificial casts and worm casts, but as far as could be measured by the methodology used, this did not lead to a higher number of micro-

aggregates. Direct estimation of the amount of micro-aggregates will be necessary in the future. However, it is likely that repeated stimulation of bacterial growth, probably in combination with drying-rewetting cycles, causes the formation of micro-aggregates over a number of cycles of cast formation. Worm casts can become colonised by fungi very quickly, and this will increase surface-related stability. The stability of surface-sterilised casts of both types, when subjected to ageing, did not increase as much as that of casts of the non-sterile treatments. This implies that the biological processes were more important than the age-hardening. This is probably because earthworm casts are too wet for thixotropic processes to occur to a significant effect. One drying-rewetting cycle produced an instant increase in stability, but the same levels of stability came about by fungus grown on casts of Mount Bold soil after 6 days. However, it can be assumed that the effects of drying-rewetting will be more persistent with time than the effect of the hyphae.

Acknowledgements

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Dispersion of clay in wet earthworm casts of different soils

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Applied Soil Ecology (submitted)

Summary

Earthworms were fed soil from two polders, differing in age and land use, permanent grassland and arable field. Casts were analysed directly or after ageing. Both sterile and non-sterile casts were used, to study the role of microbial activity in stabilisation of casts. To establish the role of polysaccharides in aggregate stabilisation, dispersability in K-periodate/K-borate was compared to that in demineralised water. Polysaccharide content was measured in untreated, water-treated and periodate-treated samples. The results for cast material were compared to field aggregates of comparable size, which were treated in the same way as the casts (sterilisation, ageing, periodate).

Ageing did not affect the stability of water-treated field aggregates. Fresh water-treated earthworm casts were very unstable. Casts from old soil were more stable than casts from young soil, and those from grassland were much more stable than those from arable soil. Field aggregates from arable soil were much more susceptible to periodate treatment than aggregates from grassland, as was the case in casts. Casts and field aggregates from old grassland soil were least affected by periodate.

Both water and periodate treated casts became more stable within the first 4 weeks of incubation. This increase in stability occurred both in sterile and non-sterile casts, so microbial activity was not necessary for stabilisation of wet earthworm casts. Polysaccharide content within treatments did not vary over time, so changes in stability of wet earthworm casts were not related to changes in amount of polysaccharide. We conclude that reorientation of clay particles, leading to closer contact between particles and/or binding substances may explain stabilisation in continuously wet earthworm casts.

Earthworms strongly increase the mobility of clay, and thereby they may also liberate organic matter that was physically protected by the clay. In wet casts, it takes a few weeks before the dispersion of the clay is reduced again, possibly enabling enhanced decomposition during those weeks. The effect of earthworms on liberation of organic matter will be stronger in relatively unstable soil.

Introduction

The size distribution and stability of aggregates are important attributes of soil structure. Aggregates are formed and broken down continuously. Newly formed aggregates are not

very stable (Mehta et al. 1960; Chaney and Swift 1986; Emerson and Dettman 1959). With time, aggregate stability often increases, but it may decrease again later. Differences in aggregate stability can only be understood if formation of aggregates and processes affecting their stability are considered separately (Allison 1968; Skinner 1986). Aggregates can be formed by shrinkage following drying, ice-lenses or roots splitting clods into smaller aggregates, tillage operations, and egestion by burrowing soil animals.

Stabilisation of a newly formed aggregate can result from different processes. Stability of aggregates is due to bonds between particles varying in size from very fine clay platelets via micro-aggregates to large sand grains (Tisdall and Oades 1982). Bonds can vary from electrostatical attraction to chemical linking (Emerson 1959).

Under wet conditions, stability may change by thixotropic processes, which involve rearrangement of the clay particles and their surrounding water films (Utomo and Dexter 1981b). If distances between clay particles are reduced, aggregate stability increases (Blake and Gilman 1970; Kemper and Rosenau 1984). Arya and Blake (1972) and Molope et al. (1985a) found enhanced stability by thixotropic changes within 3 days.

Organic products, such as polysaccharides, can stabilise aggregates, too. Microbial growth has been reported to enhance aggregate stabilisation, especially when microbes have enough substrate (Skinner 1978; Chaney and Swift 1986). The stabilising effect of microbes is usually attributed to their production of polysaccharides, which can bind together mineral soil particles. Mixing soil with microbial polysaccharides indeed increases stability of aggregates (Chaney and Swift 1986, Chapman and Lynch 1985). Roberson et al. (1991) found a positive correlation between polysaccharide content, microbial biomass C and stability of aggregates. Cheshire et al. (1984) and others studied the influence of polysaccharides indirectly by destroying the polysaccharide with periodate. Particulate organic matter can also play a role in aggregate stabilisation: by adhering to clay particles larger aggregates can be held together (Tisdall and Oades 1982).

All these processes of stabilisation after the formation of aggregates can only occur if at least part of the clay particles present are mobile, so that they can come into closer contact with other particles and binding products.

In many papers about aggregate formation and stability the role of the soil fauna is ignored. Yet, earthworms, which are large soil ingesting soil animals, can overturn up to 25% of the Ah horizon in one year (Lee 1985, p182), and thus can be major aggregate forming agents. Earthworms ingest both mineral material and organic fragments. In the gut large amounts of watery mucus are added, and the soil is kneaded thoroughly (Barois et al. 1993). In this way field aggregates are remoulded and existing bonds between particles are disturbed and clay particles are dispersed during passage. Newly formed earthworm casts are very wet and unstable aggregates. The wet state favours thixotropy. Furthermore, the casts quickly lose

some of the water by evaporation or capillary suction of the surrounding soil, which enhances their physical stabilisation. Stabilisation of wet earthworm casts may be increased by microbial activity, o.a. by production of polysaccharides, in addition to the physical effects. There is a wealth of literature about stimulation of microbial populations in soil by earthworms (e.g. review by Satchell 1967). Direct binding by fungal hyphae can also help stabilise earthworm-formed aggregates (Marinissen and Dexter 1990). Furthermore, many coarse organic fragments present in the casts may exhibit binding properties.

The relative importance of the different binding processes may alter when the casts dry, and a close contact between clay and organic material is established. Organic bonds formed after drying are generally strong, so only sturdy methods can reveal differences in aggregate stability in casts differing in amount and type of organic materials. In wet casts, changes in aggregate stability are partly due to subtle differences in the position of clay in relation to organic matter, and can be measured with relatively gentle methods.

We designed an experiment in which we tried to separate the possible stabilising processes in wet casts. We used soil from two polders (one old and one young) from fields with different land use (arable and permanent grassland). To establish the relative contribution of physical and microbial processes we sterilised half of the samples by γ-irradiation. The microbial populations in the non-sterilised aggregates were evaluated by direct counts using soil smears and selective staining for bacteria, total fungi and active fungal hyphae. The sterility of the γ-irradiated samples was checked using plate-countings. To evaluate the role of polysaccharides, aggregates were treated before analysis with either water or K-periodate/K-borate solutions. Periodate disconnects C-bonds in hydroxy-groups, borate will dissolve the remaining products (Churchman and Tate 1986; Emerson et al. 1986). Borate could also dissolve humic substances of low molecular weight (Angers and Mehuijs 1989). Most studies use the more soluble Na-salts of periodate and borate, but to minimise effects on dispersion by the cation we used K-salts instead. Furthermore, the polysaccharide content of field aggregates and worm casts was determined in untreated, water-treated and periodate-treated material.

Materials and methods

Soils used

The soils used are from two polders, reclaimed from marine sediments, one old (Bildt polder in Friesland, reclaimed around 1550) and one young (Noordoost Polder, reclaimed in 1939). In each polder, sites were chosen under two types of land-use: grassland since the reclamation and arable land. Soil material from the top 10 cm of each of the four fields was

sampled in February. The soils will be referred to as: OA (old arable), OG (old grass), YA (young arable) and YG (young grass). Some characteristics of the soils are given in Table 6.1. Both young soils, but especially YG, were lower in clay content than the old soil. In all soils except OG around 90% of the exchangeable cations consisted of Ca^{2+}. In OG this was 80%. The OG soil had much higher amounts of Ca^{2+} and Mg^{2+} at the exchange complex than the three other soils. X-ray diffraction analysis showed that all soils had similar clay mineralogy, which was dominated by illite (mica), with some kaolinite and smectite. In the old soils smectite contents were slightly higher, at the expense of illite.

Table 6.1 Characteristics of the soils used

SOIL	Texture <2μ	2-50	>50μ	pH	$CaCO_3$ (%)	CEC (meq/100 g)	Base saturation (%)	org C (%)
OA	29.9	46.1	24.0	6.0	3.9	24.1	93.4	nd[1)]
OG	34.4	41.5	24.1	5.8	2.7	37.6	96.3	6.9
YA	24.8	62.0	13.2	7.5	10.4	17.9	100.0	2.8
YG	9.5	57.3	33.2	6.9	6.1	18.2	100.0	nd[1)]

[1)] nd = non determined

Production of worm casts

After air-drying, the soil was sieved through a 2mm sieve, and slowly wetted on sandbeds to a suction of -50 cm. PVC-rings of 2.5 cm height were filled with 10 g of soil, and closed with fine netting. The rings were placed in plastic containers on a bed of coarse aggregates of the same soil type at a suction of -50 cm. Adult Lumbricus rubellus earthworms, reared on compost, were kept for four days in wetted soil of the same origin and structure (sieved <2 mm) as used later in the experiment to get them used to soil and fill their guts with soil. The soil was renewed after two days, to remove even the last compost remains.

In every ring 2 earthworms were kept for 24 hours. After removal of the worms, half of the plastic containers were sterilised by γ-irradiation (4 MRad, using a ^{60}Co-source), for 24 hours (Heijnen et al. 1988). Cast material (sampled by hand) was analysed either immediately after removal of the worms or after sterilisation, or after storage of the rings for 1, 2, 4, 8 and 20 weeks at a constant temperature of 15 °C, in dark in their closed containers. Casts from the sterile rings were collected under sterile conditions using a laminar flow cabinet.

Incubation of field aggregates

Field aggregates 3-4.8 mm in size were obtained by sieving the air-dried soil, and wetted on sandbeds to a suction of -50 cm. They were mixed with soil sieved over 2 mm, brought at a suction of -50 cm, in rings in containers on a bed of coarse aggregates. Half of the containers was γ-irradiated. All containers were stored at a constant temperature of 15 °C, in dark. Analyses were performed at day 0, and after 8 and 20 weeks of storage.

Dispersion measurements

All dispersion measurements were replicated 5 times. Two to four moist casts were weighed onto a glass microfibre filter in small sintered glass crucibles, that were placed in slightly larger beakers. Twenty-five ml of either demineralised water or a 0.02 M KIO_4-solution was carefully added to the beakers, to fully submerge the casts. After 6 h, the solution was removed by placing the crucible on a rubber ring in a funnel that was evacuated with a water pump. The same procedure was then repeated with 0.05 M K-borate solution in case of the periodate-treated samples, or with water in case of the water-treated samples for 2 h. Extraction times and concentration of solutions were according to those used by Shipitalo and Protz (1989). After the two extractions, the filters with aggregates were moved from the crucibles into small glass beakers. Carefully, 25 ml of de-mineralised water was added. Next, the beakers were left for 18 hours. Any clay dispersed from the casts was mixed into the supernatant water using a paddle stirrer (50 rpm) for 30 sec 2 cm under the water surface, so that the stirrer did not touch the casts (Marinissen and Dexter 1990). A small quantity of the suspension was pipetted off after 1.5 h at a specified depth (according to Stoke's law) to determine the clay content. The extinction of the suspension, measured with a spectrophotometer at a wavelength of 420 nm, was translated into clay content by comparison with a standard curve. This standard curve was derived from a dilution curve of a suspension of known amount of clay (determined gravimetrically). The water content of casts and field aggregates was measured each sampling day on a subsample.

The fraction of dispersed clay as percentage of the total clay present is called the dispersion index (DI). The clay content of the material was established by treating some cast material and field aggregates ultrasonically, and measuring the clay afterwards. Very stable aggregates sometimes gave negative DI-values. This is due to errors in the light transmission measurement. The DI-measurements of the first week were omitted from all analyses, because the glass filters used at that time were of a brand that swirled up during the dispersion measurements, which caused additional dispersion by disturbance of the cast on the filter.

Polysaccharide measurements

After the dispersion measurements, the content of the beakers was poured into a sintered glass funnel lined with a glass fibre filter, and the solution was pumped off by suction with a water pump. The soil material with the filter was then frozen in little plastic containers and stored at -20°C until further analysis. At each sampling occasion untreated casts were frozen too for later analysis.

For the polysaccharide analysis 0.5 ml 12M H_2SO_4 was added to the samples, after which the containers were left at room temperature for 16 h. Next, 11.5 ml of water was added, and the samples were placed in a water bath at 100°C for 5 h. The solution was then centrifuged to remove the fibres of the filters and the sand. The supernatant was analysed for neutral sugars and uronic acids using an auto-analyser according to the methods described by, respectively, Tollier and Robin (1979) and Thibault (1979), modified by adding 0.0125M tetraborate to the sulphuric acid. Polysaccharide was analysed in triplicate. Earthworm casts of all ages but only part of the field aggregates (from day 0 and week 20) were analysed for polysaccharide. The other samples were lost due to a breakdown of the freezer.

Microbial biomass

The effect of γ-irradiation was tested by plating on 10% Trypton Soya Agar; all irradiated samples had remained sterile. In non-sterile casts and field aggregates bacteria were counted in europium-chelate stained soil smears and total length of fungal hyphae was estimated in soil agar films stained with photine (Hassink et al. 1991). Metabolically active fungal hyphae were measured on membrane filters stained with fluorescein diacetate (Söderström 1977). Bacterial numbers and fungal lengths were converted to biomass as described by Van Veen and Paul (1979).

Statistics

ANOVA was used to analyse the data. T-tests were used to establish significance of differences between pairs of observations. In the Results and Discussion sections only significant differences ($P<0.05$) are mentioned.

Results and interpretation

Stability of field aggregates

The dispersion after water treatment of field aggregates was very low and did not vary over time (Fig. 6.1A+B). Differences between the four soils were negligible, indicating that age of soils, clay content or cultivation history did not influence water dispersion measured

this way. Adding periodate and borate strongly increased the dispersion, and differences between the soils appeared (Fig. 6.1C+D). Field aggregates from the two grassland soils were least affected by periodate, indicating an influence of land use on the susceptibility of aggregates to periodate. Aggregates from the old polder were significantly more stable than those from the young polder. Stability varied little with incubation time in all soils. Only in OA, the dispersion after periodate treatment was higher in week 8 than at the start and the end of the experiment, but this difference was only just significant for the non-sterile treatment (Fig. 6.1C). Sterilisation did not markedly affect the stability of either water- or periodate treated field aggregates of any of the four soils.

Stability of worm casts

Fresh, non-sterile worm casts always dispersed more strongly in water than the field aggregates of the same soil. Dispersion varied with land use and soil age: casts from grasslands were more stable than those from arable land and casts from old soil were more stable than those from younger soil. This pattern did not coincide with clay contents of the soil. Stability of the casts invariably increased strongly in the first 2-4 weeks to more or less constant levels (Fig. 6.2A).

Sterilisation of fresh casts immediately increased stability, especially in the two arable soils at day 0. During ageing in the first 2-4 weeks stability of sterile casts (Fig. 6.2B) increased to the same level as that of non-sterile casts (Fig. 6.2A), so this increase in aggregate stability cannot be explained by microbial activity.

Periodate-treatment strongly increased dispersion of fresh casts (Fig. 6.2C+D). Casts from arable soils, especially OA, were much more susceptible to periodate treatment than those from grasslands. Again, this pattern did not coincide with the clay contents of the soils. The stability increased in the first 2-4 weeks in all soils and all treatments. Sterilisation of fresh casts led to more stable aggregates in periodate, especially in casts from arable soil. In the non-sterile casts of the two arable soils the initial decrease in dispersion after periodate was followed by a significant increase after 8 weeks, especially in casts from OA. This increase was not observed for sterile casts.

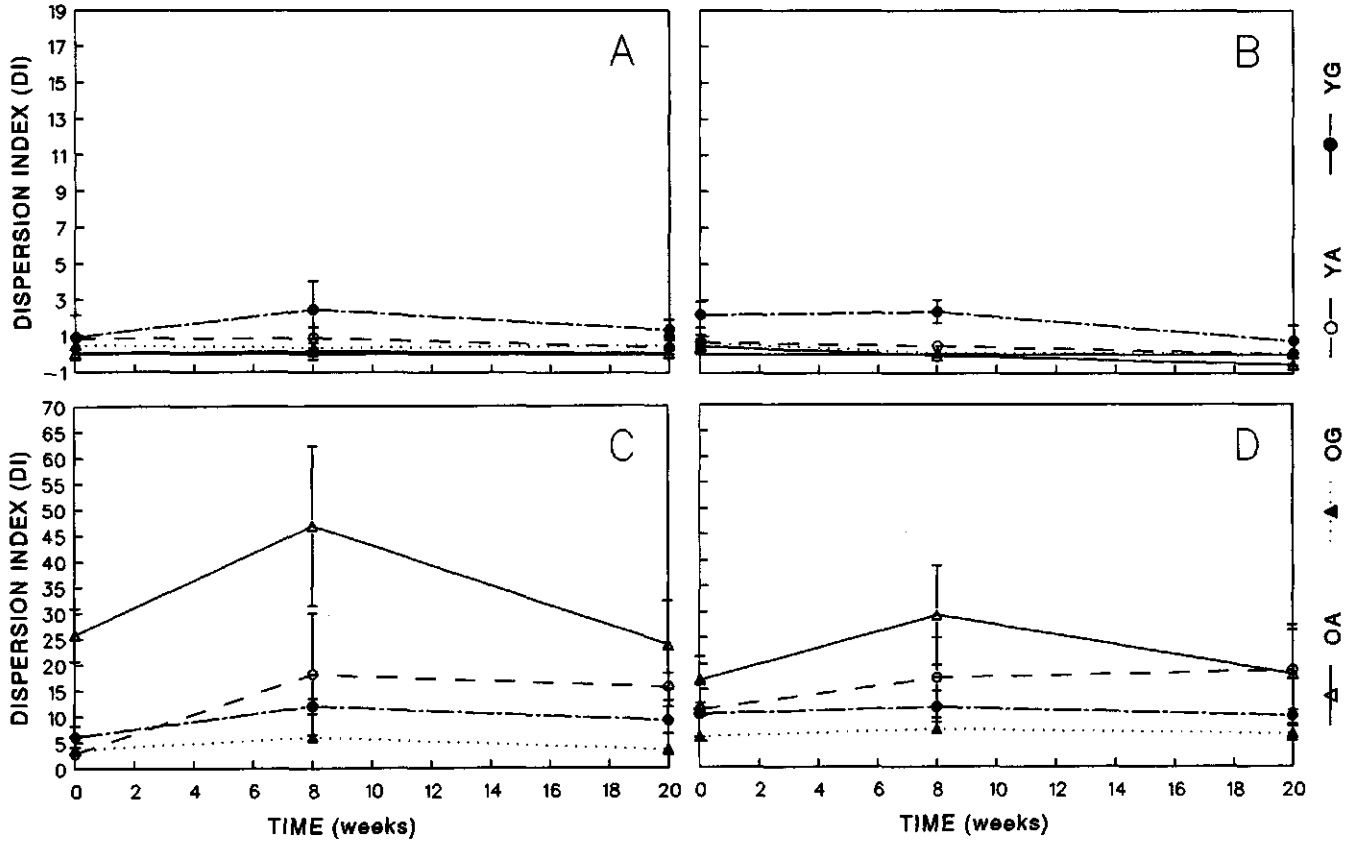

Fig. 6.1 Dispersion index of incubated field aggregates (3-4.8 mm) from the four soils against incubation time
A. after water treatment, non-sterile incubation
B. after water treatment, sterile incubation
C. after periodate/borate treatment, non-sterile incubation
D. after periodate/borate treatment, sterile incubation
Soils: OG = Old Grassland OA = Old Arable YG = Young Grassland YA = Young Arable

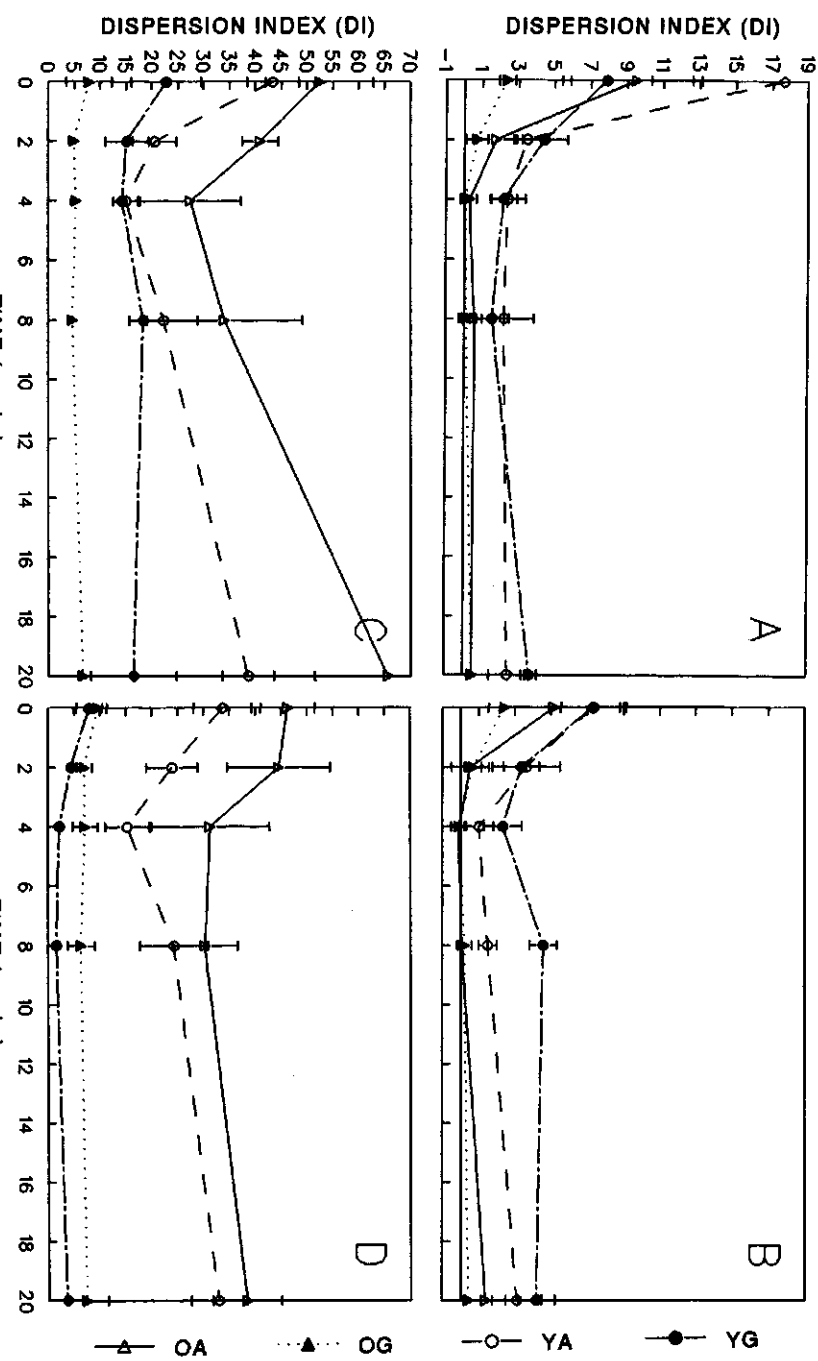

Fig. 6.2 Dispersion index of incubated earthworm casts from the four soils against incubation time
A. after water treatment, non-sterile incubation
B. after water treatment, sterile incubation
C. after periodate/borate treatment, non-sterile incubation
D. after periodate/borate treatment, sterile incubation
For abbreviations see legend Fig. 1.

Polysaccharide content

In both field aggregates and worm casts the polysaccharide content varied little with time in the different treatments and soils. The sum of neutral sugars and uronic acids (less than 4% of total polysaccharide) of field aggregates and worm casts is represented in Fig. 6.3. Total polysaccharide contents were significantly higher in grassland than in arable soils, especially in the oldest soil (OG). The low polysaccharide content in YG might be due to the low clay content of the soil. Clay particles can protect polysaccharides against decomposition (Cheshire et al. 1979). There was no significant difference in total polysaccharide content between young and old arable soil, even though the young soil was much lower in clay content than the old soil. Water treatment reduced polysaccharide contents to the same degree in the two arable soils, while YG had only slightly higher residual polysaccharide content than the two arable soils. OG retained considerably more polysaccharide after water treatment than the other three soils. After periodate-treatment much less polysaccharide remained, but again the content in OG remained much higher than in the other three soils. However, the percentage (of the amount present in untreated aggregates) remaining after water and periodate treatment was similar for all soils, viz. about 70-90% after water treatment, and 40-50% after periodate treatment.

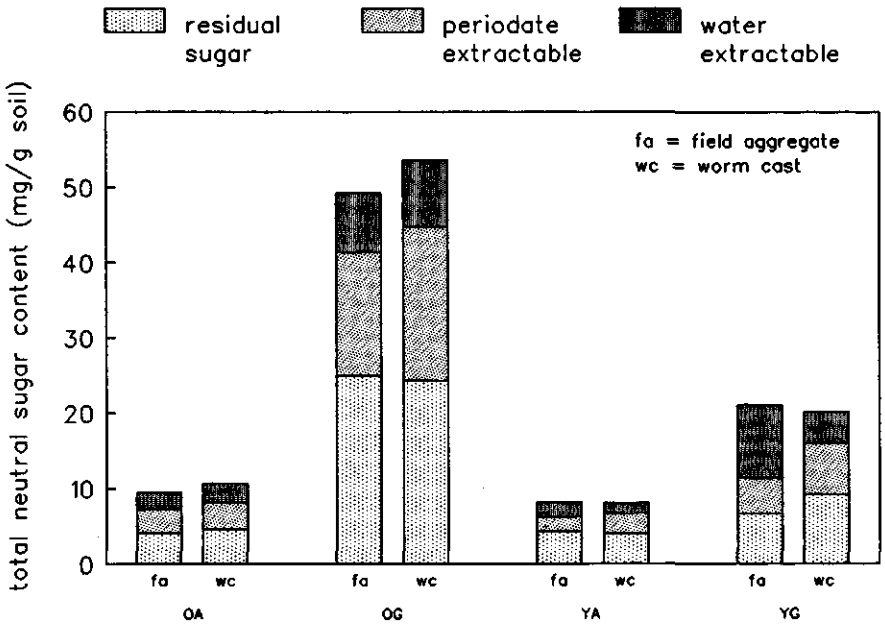

Fig. 6.3 Total neutral polysaccharide content in field aggregates and earthworm casts from the 4 soils
For abbreviations of soil names see legend Fig. 6.1

Microbial growth

The γ-irradiated field aggregates and casts remained sterile throughout the experiment. The microbial biomass of non-sterile field aggregates and casts is presented in Fig. 6.4. Of field aggregates, OG had the highest microbial biomass. The relatively low values for microbial biomass in YG might be due to the low clay content. No changes in biomass of bacteria and fungi with time were observed in any type of field aggregates. Fresh casts had microbial biomass similar to field aggregates from the same site. Microbial biomass in the casts increased in the first few weeks of incubation. In casts of OG, the biomass increased considerably to a constant level after 4-8 weeks. In OA casts the microbial biomass increased temporarily during the first two weeks, and later declined to values slightly higher than in field aggregates. In casts from YA and YG soils microbial biomass first increased and later decreased but these changes were not statistically significant. The percentage active biomass of the total fungal biomass was 1-10% initially, but peaked to 10-20% during the early weeks of the experiment in casts of all 4 soils (Fig. 6.5). Percentage active fungal biomass of all soils except OA returned to the level of field aggregates at the end of the incubation period. In OA, however, the percentage active hyphal biomass declined more strongly to values even lower than found in field aggregates. This decline in active hyphae coincided with a strong increase in the susceptibility to periodate treatment of this soil (Fig. 6.2c).

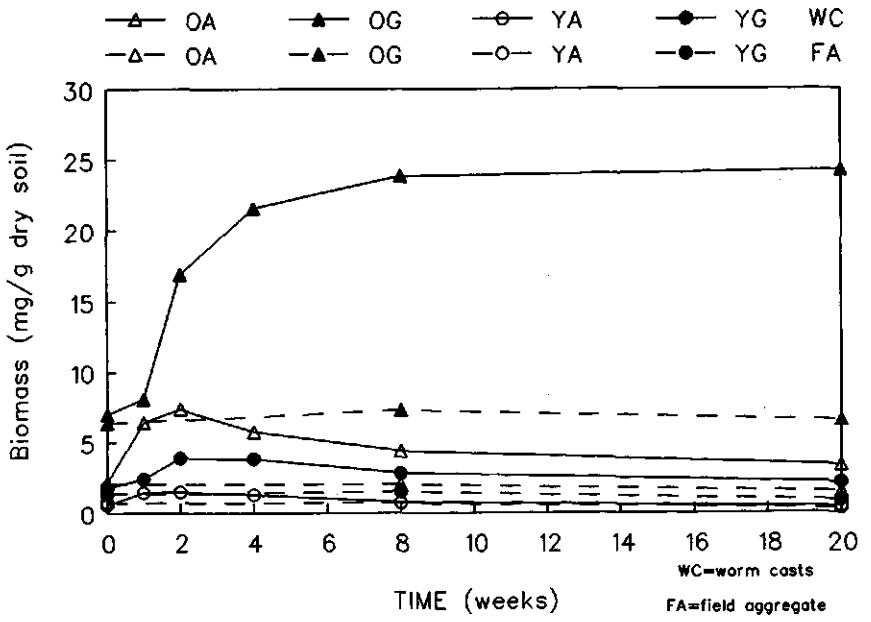

Fig. 6.4 Development of total microbial biomass during incubation of field aggregates and earthworm casts from the 4 soils. For abbreviations of soil names see legend Fig. 6.1

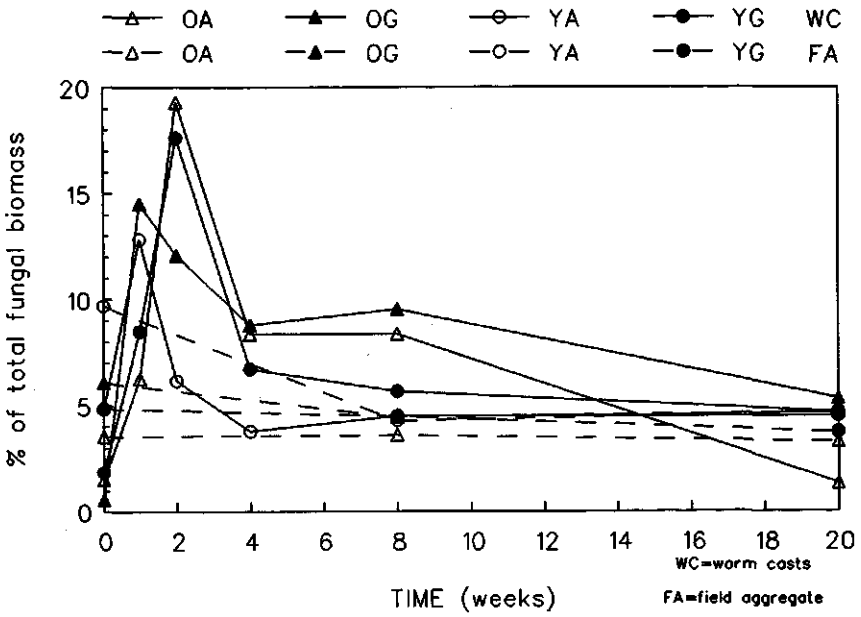

Fig. 6.5 Percentage of active fungal biomass of total fungal biomass in the 4 soils. For abbreviations of soil names see legend Fig. 6.1

Discussion and conclusions

Aggregate stability was measured in such a way, that differences between relatively unstable aggregates, like freshly formed wet earthworm casts, could be revealed. For the more stable field-aggregates, dispersion in water was not sufficiently disruptive to reveal differences in aggregate stability. However, differences between wet earthworm casts from different soils might reflect differences in stability of undisturbed aggregates (Chaney and Swift 1986), in the sense that during production of casts, earthworms can disrupt soil material with a high aggregate stability less than soil material of lower stability. Newly formed earthworm casts from old polders had the highest stability in water, and casts from grassland soil were more stable than from arable land, especially in the old polder. Marinissen and Dexter (1990) found that artificial aggregates of old soil from Australia more closely resembled worm casts than aggregates made from young Dutch polder soil. In the young soil the worm casts were much more easily dispersible than artificial casts. Hindell et al. (1994) found that dispersion was higher in artificial casts than in earthworm casts, which they related to the higher force used to make artificial casts. Earthworms can

only exert a pressure of 1.6 kPa in their gut (McKenzie and Dexter 1987). The results of all these studies indicate that earthworms can significantly mobilise clay, but more so in relatively unstable soils.

Sterilisation of fresh casts slightly increased the stability in case of grassland soils, and strongly increased stability of arable soils, at least initially. This contradicts results by Tisdall et al. (1978), who found that γ-irradiation decreased aggregate stability. Griffith and Burns (1968) attributed the loss in stability after irradiation to changes in the binding properties of the polysaccharides. In our experiment the total amount of polysaccharide did not change by sterilisation, but we have no information on the type of polysaccharide. Sterilised and non-sterilised samples had the same water content, so drying could not explain the increased aggregate stability by sterilisation. Tisdall et al. (1978) and Griffith and Burns (1968) did not wet their soil to levels as high as the very wet earthworm casts that were used in the experiment described here. Perhaps, irradiation caused a rearrangement of the dispersed clay particles in wet casts.

Aggregates are susceptible to periodate/borate treatment, for several reasons. First, the treatment destroys polysaccharides, which may bind clay particles. Hence, differences in polysaccharide contents may result in differences in susceptibility to periodate. However, absorbed K^+ from periodate and borate solutions may have caused increased clay dispersion upon bringing the samples in demineralised water before analysis. The strong drop in salt concentration could cause swelling of the double layer and increased dispersion. Shipitalo and Protz (1989) showed such a cation effect on clay dispersability even at low concentrations, but they used Na^+ which is a stronger disperser than K^+. The effect of cation and periodate will probably vary during incubation, even though the moisture content and salt concentration are kept even. If, during ageing, micro-aggregates are formed by rearrangement of clay particles, these structures can be less accessible to both K^+ and periodate.

The lower susceptibility to periodate of grassland aggregates than aggregates from arable land, is in accordance with the findings of Greenland et al. (1962) and Clapp and Emerson (1965). Field aggregates of OA were much more sensitive to the periodate treatment than those from YA, may be due to the presence of free $CaCO_3$ in YA, causing high levels of Ca^{2+}, which restricts the influence of cation/periodate-treatment (Clapp and Emerson 1965; Greenland et al. 1962). The stability of periodate-treated field aggregates did not change over time, which indicates that ageing does not influence stability in aggregates that have not recently been disturbed.

The much higher susceptibility to periodate-dispersion of fresh casts from arable soil in OA than in YA could be due to the presence of free $CaCO_3$ in YA. In this experiment, worm casts did not contain significantly more polysaccharide than field aggregates, and changes in polysaccharide content over the incubation period were insignificant. Yet, during

ageing casts became less susceptible to periodate treatment. This increase in stability occurred both in sterile and non-sterile casts. Sterile and non-sterile casts could differ in quality of polysaccharide, either through its chemical composition or the location of the polysaccharide. Harris et al. (1963) found that manually added polysaccharide was more sensitive to periodate treatment than polysaccharide that was produced in situ, probably in the form of mucigels that were encrusted, and thus protected, by clay particles (Foster 1985). However, the increase in stability of periodate treated casts also highly resembled the pattern of development of stability in water treated earthworm casts. This indicates, that the increase in stability was mainly due to a closer rearrangement of clay, while increased binding by polysaccharides did not play a role in the wet state of the casts. The rearrangement of clay would lead to lower sensitivity to both the K^+ and the periodate.

Many authors (e.g. Griffith and Jones 1965; Harris et al. 1963 and 1964) state that microbial effects are important for the stabilisation of aggregates. However, in most of these papers, aggregates were dried before analysis. Blake and Gilman (1970) did not find a difference between stability development in wet artificial aggregates under sterile and non-sterile conditions. We also did not find evidence for stability increase due to microbial activity in wet earthworm casts. The results presented in this study do not necessarily mean that (microbial) polysaccharides do not play a role in stabilising earthworm casts. Whether or not earthworm casts are dried may modify the predominance of one or more of the possible stabilising processes. E.g., purely thixotropic changes are only possible as long as the casts are wet, while drying may be necessary for the organic material (both particulate organic material and polysaccharides) to form bonds. Kladivko et al. (1986) found a strong positive correlation between the numbers of earthworms per pot and the stability of aggregates if the aggregates were analysed wet. After drying, aggregate stability was mainly determined by the type of plant remains, although the effect of earthworms was still significant. Shipitalo and Protz (1989) hypothesised that drying increased the formation of bonds through organic material, partly in the matrix (influence of polysaccharides), but, in their view, mainly through coarse plant debris that links the internal parts of the aggregate together. The type of particulate material remaining from different food types influenced the increase in stability. For example, casts of *Lumbricus terrestris* contained fewer plant remnants and had a lower stability than dried casts from *L. rubellus*. Binding through coarse organic debris is also known from other studies: Churchman and Tate (1986) and Roberson et al. (1991) found larger differences in organic matter amended treatments using dried aggregates than when using wet aggregates.

The low clay contents in the young soils, especially in YG, did not seem to influence the pattern of dispersability. It seemed to influence the amount of microbes present and/or active and the amount of polysaccharide present, but these factors did not affect development of stability in wet casts.

Whatever process influences the stability of aggregates, an important prerequisite is that the clay present is able to move and rearrange itself around binding organic material. Earthworms, by mixing soil with watery mucus in their gut, are efficient in mobilising clay, and they also mix in organic matter so that new binding sites are created. Doing this, they may influence organic matter dynamics: in their gut physically protected organic matter is freed from the clay particles, and remains unprotected for a while in wet casts, thus potentially enhancing mineralisation. After drying a larger part of the organic matter present is occluded and is thereby protected from quick disintegration in the highly stable dry casts. In earthworm casts of grassland soil the clay was less mobile than in those from arable land, which may mean that the more stable aggregates are present, the less clay, and thus also occluded organic matter, can be mobilised by worms. However, in worm casts from all soils the clay was much more mobile than in uningested soil during the first two weeks after formation. The dispersed state of the clay may facilitate mineralisation in fresh wet casts of all types of soil, but the effect of earthworms on decomposition will be highest in unstable soils.

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Earthworm induced distribution of organic matter in macro-aggregates from differently managed arable fields

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Abstract

To study the influence of soil structure on organic matter decomposition, and the possible role of earthworms therein, aggregates of the size of earthworm casts (3-4.8 mm) were sieved from air-dry soil of three arable fields. Due to different management histories (in terms of manuring, ploughing and pesticide use) organic matter contents and earthworm population densities varied markedly between the fields. The fraction of aggregates that withstood wet sieving was determined and collected. Organic C content and the short-term C-mineralisation rate of the organic matter was measured after grinding in the dry-sieved total 3-4.8 mm fraction and in the stable aggregates, and calculated for the unstable aggregates. C-mineralisation of sonicated samples was used to evaluate effects of physical protection of organic matter.

The percentage of water-stable aggregates increased with earthworm numbers, though stable aggregates were also found in the field without earthworms. In all cases, stable aggregates of all fields had significantly higher organic C-content than the total fraction. The C-content in stable aggregates from the field with high organic matter inputs was significantly higher than from the two fields with low organic inputs. These two fields did not differ in C-content of stable aggregates, even though they only shared the same treatment in the last 5 years, after 35 years of different organic management. On the other hand, after only 5 years of different management, fields that had shared 35 years of identical high organic matter input levels differed highly in earthworm numbers and C-content of stable aggregates.

Organic matter appeared to be better protected in stable than in unstable aggregates. Yet, mineralisation was higher in the ground material from stable than from unstable aggregates, at least in the conventionally managed fields with no or few earthworms. However, in the field with highest organic matter inputs and earthworm numbers, the decomposability of the organic matter in stable and unstable aggregates was comparable. The reasons for this remain unclear. Differences between the aggregate fractions of the three fields in decomposability might indicate that in the integrated field stable aggregates form more quickly or persist longer than in the conventionally managed fields.

This study provides strong indications, but no proof, that earthworms stimulate these processes of C-enrichment and stabilisation of macro-aggregates.

Introduction

Soil organic matter is not a homogeneous material, but a mixture of different fractions, each with its own decomposition rate (e.g. Parton et al. 1987). The origin and real nature of these fractions is still unknown, but can be associated with either the chemical composition of the material or with physical protection, e.g. by associations between clay and organic matter (Van Veen et al. 1985). However, also soil macro-structure might exert an important influence on decomposition rate, depending on the precise location of the organic matter in the soil profile and within the soil structural elements (Beare et al. 1994a and b). Organic material can be protected against decomposition when it is positioned in pores too small for bacteria to enter (protected pore space, Postma and Van Veen 1990), or it can be inside large aggregates, which can become partially anaerobic by slow oxygen transport through the small intra-aggregate pores (Rappoldt 1990). The protection will only exist as long as the aggregate remains intact, so stable aggregates will provide a higher degree of protection than unstable ones. Protection in stable aggregates might further be promoted by greater abundance of organo-mineral complexes (Shaw and Pawluk 1986), through close contact between clay and organic material in these complexes. The organic C that is preferentially incorporated in stable aggregates might also differ in decomposability from the rest of the soil organic matter (Elliott 1986, Beare et al 1994 a and b).

In the process of incorporation of litter into the soil aggregates, earthworms play an important role. They are often the dominant soil ingesting animals. Earthworms can destroy and form organo-mineral complexes in their gut (Barois et al. 1993). After excretion of the casts the formation of new micro-aggregates still proceeds (Marinissen et al., submitted). Earthworms also influence macro-aggregate stability (Shipitalo and Protz 1988; Marinissen and Dexter 1990). Marinissen (1994) found a strong correlation between macro-aggregate stability and actual earthworm numbers, using a range of fields from a Dutch polder, including those used in the experiment described in this paper. Aggregate stability is a transient soil characteristic (Kandeler and Murer 1993, Marinissen 1994). Aggregate stability therefore quickly reacts to changes in earthworm density: after suppressing earthworms for one year (Blair et al., in press), Ketterings et al. (1993) found significant reductions in stability of large aggregates. Earthworm casts contain more organic matter than uningested soil (Shipitalo and Protz 1988). Earthworms also enhance aggregate stability. Therefore, in the present study we hypothesised that a higher fraction of the soil organic C pool will be incorporated in stable macro-aggregates with increasing earthworm activity. This would also lead to a higher proportion of the organic matter that is, at least temporarily, protected from decomposition.

Methods

In March 1992, soil samples were collected from the top 5 cm of three arable fields differing in management history. The soil was a calcareous silt loam (Typic Fluvaquent), with a pH of 7.5 and organic matter contents varying between 2.2 and 2.8 % depending on management. For 35 years fields either received organic manure (Block A) or NPK fertiliser only (Block B). Then, 5 years before sampling, on half of each block, management was changed to 'conventional' (medium organic matter input, consisting of crop- and intercrop-remains, high level NPK-fertiliser, chemical pest- and weed-control) or 'integrated' (high organic matter input, consisting of crop- and intercrop-remains plus manure or compost, reduced NPK-fertiliser and reduced chemical weed- and pest-control). For details of the management see Lebbink et al. (1994). As a result of these differences in management, at the time of the study, earthworms were absent in the B-block (referred to as Conv-B), very numerous (maximum of 700 individuals per m^2) in the integrated field on the A-block (Int-A) and low in numbers (maximum of 20 per m^2) on the conventional field on the A-block (Conv-A) (Marinissen 1992 and 1994). In the Int-B field, earthworms were starting to colonise (Marinissen and Van den Bosch 1992), the irregular earthworm distribution kept us from sampling this field.

The soil was air-dried in the laboratory and sieved into different aggregate size classes. In October 1993, part of the 3-4.8 mm fraction was separated into 'stable' and 'unstable' aggregates by wet sieving (prewetting of 40 g dry aggregates with a plant sprayer, sieve size 20 cm diameter, 3 mm mesh-size, sieving time 3 minutes, strokelength 3 cm, frequency 0.5 cycle per second). The stable aggregates remaining on the sieve, were dried at 40 °C for 48 h (found to give comparable results to 24 h at 105 °C) and ground to pass a 0.5 mm sieve. Another part of the 3-4.8 mm fraction was prewetted in the same way, and submersed for 3 minutes, but not sieved, and also dried and ground. C- and N-content were measured using a Carlo-Erba combustion C/N-analyser after removal of carbonate-C with HCl (Nieuwenhuize et al., in press). For mineralisation measurements, we used ground material to prevent that small differences in mean aggregate size between the fields would influence the mineralisation. For each sample, 3.5 g of air-dry material was wetted to just below saturation in 130 ml closed serum bottles with a septum in the lid. To find the amount of water needed, water was added to a spare sample until ponding occurred. The samples used in the experiment were wetted with slightly less water. After 1, 2, 8, 17 and 23 days, CO_2 in the headspace was sampled with a syringe and measured using gas-chromatography (Becker 722 with a silica gel column, 50 m * 0.25 mm, thermal detector). If the CO_2-level in the bottles reached 1%, the headspace gas was replaced by ambient air of known CO_2-content. After 49 days 20 ml of demineralised water was added to the

bottles, and the samples were sonicated using a 600 Watt ultra-sonicater (at 40% of total power) with a stepped 3 mm microtip during 40 seconds with intervals, so that pulses were given for 70% of the time. C-mineralisation of the sonicated samples was measured over a period of 11 days. Mineralisation both before and after sonication is presented as percentage of organic C present at the start of the incubation.
Statistical analysis was done using ANOVA with additional T-tests to test differences between pairs of observations ($P<0.05$). C-content and the quantity of potentially mineralisable C in unstable aggregates was calculated as follows:

$$Cu = \frac{Ct - s * Cs}{(100 - s)}$$

in which
Cu = C-content or mineralisation of unstable aggregates
Ct = Mean C-content or mineralisation of total fraction 3-4.8 mm aggregates
Cs = C-content or mineralisation of stable aggregates
s = % of stable aggregates

Because the samples of the total fraction and those for stable aggregates, were not dependent, no statistical analysis could be performed with data on unstable aggregates. However, if significant differences occurred between stable aggregates and the total fraction, this means that also unstable aggregates will differ significantly from stable aggregates. When significant differences between stable and total fraction occurred, we will relate this to the difference between stable and unstable aggregates in the following text.

Results and interpretation

In all fields, also in the absence of earthworms (Conv-B field), stable aggregates were present. However, aggregate stability significantly increased with increasing organic matter inputs and increasing density of earthworms: in Conv-B only 2.9±1.3 % of the 3-4.8 mm fraction was water-stable, against 8.1±2.2 in Conv-A and 12.1±2.4 % in Int-A. The organic C-content of the total fraction was lowest in Conv-B and highest in Int-A (Fig. 1). In each field stable aggregates had significantly more C than unstable aggregates. The C/N ratio of the organic matter in the different aggregate fractions did not differ (data not shown). C-content of stable aggregates was significantly higher in Int-A than in the other two fields.

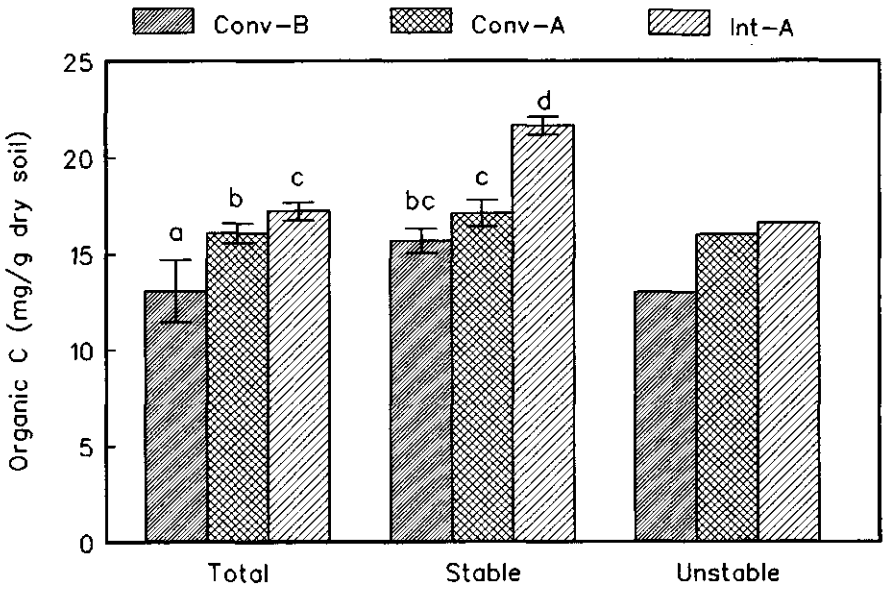

Fig. 7.1 Organic C-content of macro-aggregates (3-4.8 mm) from three different fields differing in management-history. Int-A:Organically managed for 35 years, followed by 5 years of 'integrated' management (for details see text). Conv-A: Organically managed for 35 years, followed by 5 years of 'conventional' management (for details see text). Conv-B: NPK-fertilised only for 35 years, followed by 5 years of 'conventional' management (for details see text)

The differences in aggregate stability and C-contents of stable aggregates led to large differences in the distribution of the total C contained in the 3-4.8 mm fraction over stable and unstable macro-aggregates in the three fields: in Conv-B only 3.5% of the total C in the 3-4.8 mm fraction was in the stable aggregates, the rest in unstable, whereas it was 9% and 15% for Conv-A and Int-A respectively.

C-mineralisation, expressed as the mass fraction (%) of the C originally present, did not differ significantly between stable aggregates from the three fields (Fig. 7.2). Mineralisation of C from stable aggregates was significantly higher than that from unstable aggregates in two of the three fields (Conv-B and Conv-A; Fig 2), but in Int-A stable and unstable aggregates had the same C-mineralisation.

After ultrasonic treatment, mineralisation rates were much lower than before (data not shown), probably because anaerobic conditions occurred in the fully submerged soil material. Therefore, it is not possible to compare decomposition before and after sonication directly. However, ratios within treatments can be compared, e.g that between mineralisation in stable aggregates and total fraction. The ratio of mineralisation of stable and total fraction invariably increased by sonication (Table 7.1). These ratios should be

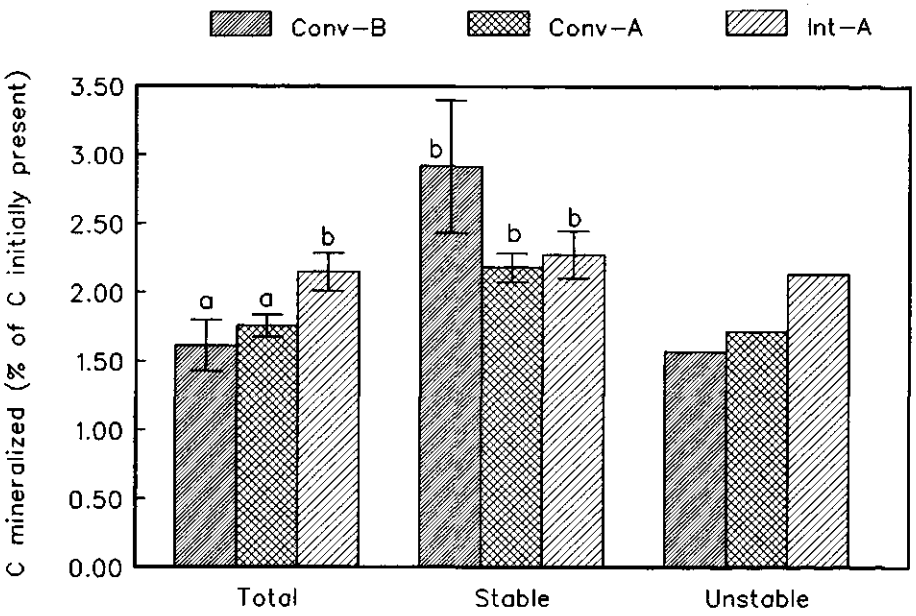

Fig. 7.2 C mineralised after 23 days of incubation as % of total organic C present in air dried and ground macro-aggregates (3-4.8 mm) from three different fields differing in management-history. For abbreviations of field names see legend Fig. 1. Total: total fraction of macro-aggregates (only wetted). Stable: aggregates retained on the sieve after wet-sieving. Unstable: calculated mineralisation from aggregates fallen through the sieve during wet sieving.

interpreted with great care, because the sonicated samples yielded highly variable results. The most certain conclusion from Table 7.1 is, that before sonication there was no significant difference between stable and unstable aggregates form field Int-A, while after sonication a significant difference occurred. The results indicate that sonication stimulated mineralisation more in stable aggregates than in the total fraction. Because sonication destroys bonds between clay particles and between clay particles and organic matter, the stimulation of mineralisation by sonication is probably due to liberation of physically protected organic matter. This indicates that physical protection of organic materials played a larger role in stable than in unstable aggregates.

Table 7.1 Ratio between mineralization of stable and total fraction of macro-aggregates (3-4.8 mm) from three different fields differing in management-history, both before and after sonication.

Field	Ratio Stable/Total	
	Before sonication	After sonication
Conv-B	1.82	3.70
Conv-A	1.25	1.35
Int-A	1.06	1.39

Discussion

We are interested in the role of earthworms in aggregate formation and organic matter decomposition. The fields studied in this experiment differed in past and present organic management, and in earthworm numbers. Organic amendment in itself can also influence aggregate formation and stabilisation. As earthworm numbers are associated with organic management, it will always be difficult to separate the influence of earthworms from changes induced by organic matter levels alone in field experiments. However, some tentative conclusions about earthworm influences can be drawn from our data.

In all fields, regardless of the presence of earthworms, stable aggregates occurred that were enriched in organic C content. However, under integrated management, where earthworm density was highest, stable aggregates not only were more abundant, but also had higher C content than in the other two fields, leading to a higher proportion of the C included in stable aggregates. The differences between the two fields on Block A were due to only 5 years of different organic management, while 35 years of management history separated Block A and Block B before the 5 years of identical management. Still, the organic matter content of stable aggregates from Int-A was significantly higher than that from both conventional fields and earthworm numbers differed widely between Int-A and Conv-A. Higher organic matter inputs by itself could also lead to enhanced aggregate stability, but we would not expect a change in organic matter content in stable aggregates. It seems likely that earthworms play an important role in increasing the organic matter content of stable aggregates. In the field study of Beare et al. (1994b) stable aggregates in a no-tillage field (NT) were also higher in organic matter content than those in conventional tilled fields (CT), while earthworm populations too were much higher in NT than in CT (Parmelee et al. 1990). The organic matter input was the same in both tillage systems.

Organic matter was better protected against mineralisation in stable than in unstable aggregates, as was indicated by the effects of ultra-sonication. Surprisingly, without sonication, we found higher mineralisation per unit of C in stable than in unstable aggregates. To exclude effects on mineralisation due to mean aggregate size (that might differ between field), we had ground the aggregates to uniform size. The fact that stable aggregates from all fields resembled each other so much in decomposability may indicate that decomposability is related more to aggregate stability than total C-content. The stable aggregates from both tillage treatments of the study of Beare et al. (1994a and b) also had exactly the same decomposability per unit of C when size effects were excluded by grinding.

Unlike unstable aggregates from the other two fields, unstable aggregates from field Int-A had the same C-decomposability as stable aggregates. We suggest the following explanation

for the observations on different C-mineralisation rates over different fractions. Unstable aggregates can be considered to be derived in part from stable aggregates by partial decomposition of certain organic matter, whereby the decomposability of the remaining organic matter is reduced. If few stable aggregates are formed, or if stable aggregates quickly lose their stability, unstable and stable aggregates will differ relatively more in decomposability of organic C. Thus, in Int-A probably stable aggregates are formed at a higher rate, or persist longer, as indicated by the small differences in decomposability of the C. If this mechanism would be valid, this would imply that the turnover time of aggregates and the soil organic matter contained in them is influenced by the management, presumably through earthworm activity.

For further evidence of the impact of earthworms on the distribution of carbon and the stability of aggregates in soils earthworm manipulation studies are very instructive. The same trends in C-enrichment in the stable macro-aggregate fraction as found in the present study were observed in the earthworm manipulation study described by Blair et al. (in press). The effects could only be attributed to earthworm activity (Ketterings et al. 1993). The results from our study shows that combining aggregate separation, organic C measurements and mineralisation studies, forms a useful methodology to apply in this type of experiments.

Acknowledgements

The authors are indebted to N van Breemen, M Beare, P Bohlen and L Brussaard for valuable comments on earlier drafts of the manuscript.

General discussion and conclusions

Introduction

The earliest record on the positive influence of earthworms on soil I came across was written by Gilbert White, in his letter to the honourable Daines Barrington on May 20, 1770 (White 1789). Since that time, Darwin (1881) referred to earthworms as the plough of nature. A special symposium on earthworm ecology was first organised in Grange over Sands in 1981. Interest in the role of earthworms in agriculture is still increasing all over the world (Germany: Becher and Kainz 1983; Joschko et al. 1989, Denmark: Christensen et al. 1987, Australia: Baker et al. 1992, France: Fayolle et al. 1994). The Swedish Arable Land Project (1980-1986) included a study on the role of earthworms in nutrient cycling (Boström 1988). An earthworm manipulation experiment considering the role of earthworms for nutrient cycling and organic matter dynamics in three different types of agro-ecosystems still continues (Blair et al, in press, Bohlen et al, 1994, Ketterings et al, in press). In the Horse-Shoe Bend tillage experiment, the role of earthworms in nutrient cycling has also been investigated (Hendrix et al. 1987; Parmelee et al. 1990).

Most studies on earthworm ecology start in the field, describing population densities in a range of treatments, or tracking population development (Boström 1988; Lofs-Holmin 1982a). A very extensive study of population dynamics was performed in a German forest (Judas 1989; Scheu 1990). With the field studies, understanding of the factors that determine population growth increases, by the use of elaborative multifactorial analysis and ordination techniques. However, quantitative knowledge of the influence of environmental factors on population growth is still scarce. Only few laboratory studies on those relations with other species than *Eisenia fetida* exist (Boström 1988; Daniel 1992; Lofs-Holmin 1980, 1982b). Problems with laboratory studies on soil inhabiting earthworms often arise because it is difficult to keep them confined in pots without upsetting their natural behaviour. Special emphasis on techniques to enable laboratory studies is needed. Modelling will be a rewarding approach in the further development of our understanding of population dynamics. The first steps were taken by Martin and Lavelle (1992) for tropical earthworms and by Daniel (1992) for the temperate area species L. terrestris. In order to asses the effects of toxicants on earthworms at the population level, a series of simple models is being developed at the IBN-Arnhem with promising results (e.g. Klok et al., in press). In this thesis, earthworm ecology formed only part of the interest, so we could not invest in large laboratory studies. Thereabove, because data on actual earthworm numbers were also

needed to relate them to soil characteristics of the field, I also opted the field sampling-statistical analysis approach.

The emphasis on the influence of earthworms on field aggregates, as described in this thesis, is relatively rare in aggregate research. Most studies about aggregate formation do not consider the effects of earthworms. The work of Shipitalo and Protz (1988 and 1989) is confined to laboratory, not field, experiments with earthworms. Basic work on aggregate formation theories are the papers of Tisdall and Oades (1982) and Oades (1984). The first to use this theory and apply it to soil organic matter dynamics was Elliott (1986) in his paper on native grassland and arable fields. Also in his model of aggregate dynamics the role of soil fauna remains unmentioned. Recently the relation between aggregates and soil organic matter received due attention in the Horse Shoe Bend sites (Beare et al. 1994a and b), but, again, the role of soil organisms in these processes is only briefly mentioned.

In this chapter first data on influences of management practice on earthworm populations are discussed. Then the focus will switch to aggregate formation and stabilisation by earthworms. As a conclusion, a hypothetical outline of the causal links between aggregate dynamics and organic matter turnover, which explains why earthworms are so important to organic matter dynamics.

Earthworm populations and management practice

In general, relatively high numbers were found in the integrated fields (chapter 3). At the start of the new management earthworms had just colonised the whole experimental A-block (with the former organic matter rich management) (chapter 2.1). It seems therefore logically to assume that their densities then were probably the same all over the A-block. However, after 5 years of conventional management on the A-block earthworm numbers were much lower than those in the integrated fields (chapter 4). Probably, earthworms had declined by a combination of pesticide effects and lower organic matter inputs.

Of the pesticides, especially fumigants against nematodes are very deleterious for earthworms (Edwards and Lofty 1978; Marinissen, unp results). The two conventional fields were treated with nematicides two times during the first five years of new management. The effect of fumigation may partly have been indirect, through reductions of population densities of other soil fauna, especially protozoa (Bloem et al. 1994; Bouwman and Zwart 1994), which are considered important food for earthworms (Miles 1963; Rouelle 1983).

The total inputs of organic matter, which consisted of manure and crop remains, were not extremely different between 'conventional' and 'integrated' management. C-inputs were estimated to be 6200 kg C per ha in the conventional fields during the 1988-1991 crop rotation cycle and 9510 kg C per ha in the integrated fields in the same period (Van

Faassen and Lebbink 1994). The differences in organic N inputs were much larger: addition of organic N to the integrated were twice those to the conventional fields (456 against 290 kg N per ha) (Van Faassen and Lebbink 1994). Earthworms use part of the fresh organic input directly for food, and the higher nutritious value of the organic matter input in the integrated field may have increased their food availability. Their food may also have been increased because inputs of well palatable organics provide a higher growth of microbes and protozoa. Microbial biomass was 1.5 times higher in integrated fields on the A-block than in conventional fields on the B-block, but this difference was not statistically significant (Bloem et al. 1994). However, protozoan biomass was significantly higher in IntA than in ConvB (Bloem et al. 1994). It thus seems reasonable to assume that food conditions were (much) better in integrated than in conventionally managed fields. The growth rate of earthworms is strongly influenced by food quality and quantity (Boström and Lofs-Holmin 1986; Lavelle et al. 1989; Shipitalo et al. 1988). Klok et al. (in press) found in a model study that the rate of growth in the juvenile stage is the most important factor determining population growth. If environmental stress strongly retards juvenile growth, juveniles will fail to reach the reproductive phase. In almost all samplings, juveniles were the most numerous component of the population (chapter 3).

It is doubtful that earthworms have benefitted greatly from the reduction in tillage depth in the integrated fields. The species present in the fields are not very sensitive to tillage, and might even benefit from the larger amount of incorporated food (Edwards and Lofty 1982). The earthworm fauna in the integrated fields, though present in high numbers, is, however, ecologically incomplete: anecic species such as *Aporrectodea longa* and *Lumbricus terrestris* are missing. The reason for the absence of anecic species in the Lovinkhoeve soils is their low growth rate potential under the ambient tillage and food conditions. Conventionally tilled arable land is unfavourable for anecic species because the inversion tillage (1) incorporates their food (litter present at the soil surface) into the soil, it (2) scatters their underground food stores and (3) destroys their permanent living burrows. The low food inputs and high level of disturbance reduce growth rate, which is an important parameter determining colonisation success (chapter 2.2). If grassland with anecic species is ploughed, they might survive for a longer time, but population densities will be prone to decline. In a former grassland plot at the Lovinkhoeve, *A. longa* was still present after 5 years of conventional arable farming, but at low numbers (chapter 2.1). An earthworm manipulation experiment at Ohio State University was laid in a field with originally high earthworm density, including large numbers of anecics (Ketterings et al. in press, Bohlen et al. 1994). After 3 years of conventional tillage, quite a number of *L. terrestris* had survived (Bohlen et al. 1994). The survival of anecic species might be improved with reduced tillage, especially non-inversion tillage or no-tillage (Parmelee et al. 1990), which would leave much organic debris at the soil surface. However, in Dutch crop rotations also

practised in the Lovinkhoeve experiment, potatoes and sugar beets are cropped every four years. These crops necessarily involve severe physical disturbance of the soil, and thus the destruction of anecic burrows.

A major conclusion from this thesis is, that management can very quickly affect earthworm population size: reductions can occur within a few years of adverse management (chapter 4), while adding manure can lead to very high populations within one wet year in the absence of soil fumigation (chapter 3). Even relatively small negative effects of agricultural management on earthworm populations can lead to strong reductions in earthworm numbers, because they may be supplemented by adverse weather conditions. E.g. if a summer drought has decimated population, as happened in 1986 (chapter 3), and the survivors are successively killed by tillage operations in the following autumn, populations can be decimated easily.

Earthworms and general aspects of soil structure of the Lovinkhoeve Farm

The 35 years of different organic matter treatment have led to morphological differences in soil structure of the top layers of the two different blocks of the Lovinkhoeve. The different top soil structures caused differences in physical characteristics as measured under standardised conditions in the laboratory (De Vos et al. 1994). That earthworms were for a large part responsible for these differences can be deducted from the micromorphological study of Boersma and Kooistra (1994). At the start of the new management, the total porosity of the top soil of the organically manured A-block was 6.4 % as compared to 4.1 % in the inorganically fertilised B-block which had never received manure (Kooistra et al. 1989). Since then, in the Int-A field soil faunal macroporosity (of which earthworms were an important part) increased over the years (Boersma and Kooistra 1994), and had not yet reached a steady state in 1990, while in Conv-B much less changes occurred. This is in accordance with the findings in this thesis that earthworm populations still tended to increase in the Int-A field.

Root growth was also affected by the type of macropore structure present in the fields. In the conventional system roots tended to make their own pores, as shown by the higher root-soil contact, while they more often used existing pores in the integrated system (Van Noordwijk et al. 1993). In soils with high soil-root contact oxygen supply can become limiting under wet conditions, while water and nutrient uptake can become limiting for roots following existing pores (De Willigen and Van Noordwijk 1989). In the specific Lovinkhoeve situation where excess water is more often a problem than drought (De Vos et al. 1994), earthworm may have improved conditions for root growth through the formation of continuous macro-pores.

Earthworms and soil aggregates

Slaking was considered a potential stability problem of the silty soils of the Lovinkhoeve. Furthermore, slaking is an aspect of stability that can be influenced by differences in organic matter amendments. For the experiments described in this thesis therefore the wet sieving method was selected to elucidate developments in aggregate stability following the changes in agricultural management systems. In case of experiments with only small amounts of samples, dispersion measurements were made. The two types of aggregate stability measurements are closely linked (Pojasok and Kay 1990). Because of the size of their casts, earthworms should influence mainly aggregates larger than 2 mm. Therefore, this size class interested me the most. To make specific measurements on the macro-aggregates, I wet sieved size fractions of the soil that were first obtained by dry-sieving the whole soil. In this way, stability data refer to that part of the soil, that is most likely influenced by earthworms. It is an important part of the soil because macro-aggregates play a major role in determining seed-bed quality (Braunack and Dexter 1989).

This thesis, supported by literature data, clearly shows that earthworms indeed influence soil aggregate stability of the macro-aggregate size. Fresh earthworm casts, however, are

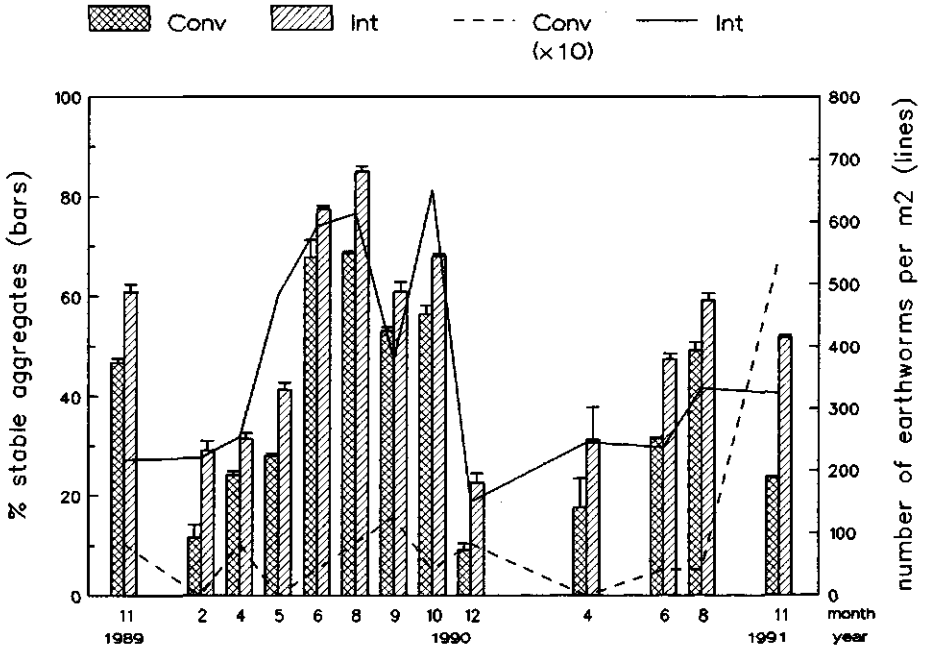

Fig. 8.1 Aggregate stability and earthworm numbers in fields of the A-block (former organic management from the Lovinkhoeve (The Netherlands) CONV = Conventional management INT = Integrated management

very unstable (chapter 5 and 6, Shipitalo and Protz 1988). During ageing they become more stable, but drying improves the stability most (Chapter 5, Shipitalo and Protz 1988). After air-drying, earthworm casts are much more stable than field aggregates. The link between earthworm populations and aggregate stability is illustrated by Fig. 8.1 (after data from chapter 4). Ketterings et al. (in press) sampled an earthworm manipulation experiment, and already found an influence of manipulation treatments on aggregate stability one year after the start of the experiment: aggregate stability was lowest in fields with reduced earthworm numbers and higher in fields with ambient and increased earthworm numbers (Fig. 8.2). This strongly indicates a causal link between actual earthworm activity and aggregate stability. The short life-time expectancy of stable aggregates (in the order of months) indicates that earthworm activity must be maintained at high levels to sustain high aggregate stability.

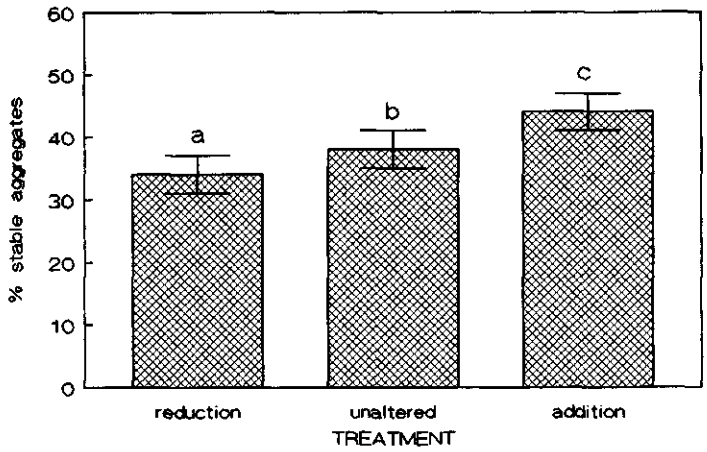

Fig. 8.2 Stability of aggregates 4-10 mm in fields with manipulated earthworm numbers. (From Ketterings et al., in press.)

In the Lovinkhoeve fields, aggregate stability was not increased enough by earthworms to prevent slaking completely. Soil crusting occurred in both fields, but was never so severe that seedling emergence was hampered. The effects of stable casts produced at the surface is counteracted by the practice of inversion tillage. In wet years, the lack of drying of the surface casts can also play a role, because wet casts are less stable than uningested surface aggregates. However, general stability conditions are still improving in the Lovinkhoeve: in 1985 in both conventional and integrated fields, continuous pores were blocked to the same amount by infillings of surface soils (Kooistra et al. 1989), but in 1987 and in 1990 much less internal slaking occurred in Int than in Conv (Boersma and Kooistra 1994). This might well be related to the increasing trend in earthworm populations in IntA.

Earthworms could also have a long-term influence on soil structure stability. A method to evaluate such stability is the determination of the LPL (Lower Plastic Limit according to Atterberg). The LPL did not vary over the season and is clearly a more invariant parameter than aggregate stability as determined by wet-sieving (chapter 4). In long-term studies of soil structure development, it is difficult to separate the effects of earthworms from those of organic matter. Soils with high organic matter inputs harbour large earthworm populations, and have higher organic matter contents than soils with low inputs. The organic matter content for different arable fields of the Lovinkhoeve was related to the LPL (chapter 4), but not to actual earthworm numbers. Actual earthworm density is not a good indicator of the total possible influence of earthworm activity on LPL, if simply because it is not even a good indicator of earthworm numbers in the recent past. In a series of old grassland soils in polders varying in age between 150 and 700 years LPL of macroaggregates was also only related to %C and not to age or earthworm numbers (Marinissen et al., manuscript). Thin section analysis indicated however, that earthworm activity was the main process responsible for the formation of A1-horizons and soil aggregates by mixing of soil material and organics.

Earthworms and soil organic matter dynamics

It is generally accepted that excrements of soil fauna potentially influence organic matter turnover (Martin and Marinissen 1993). In this paragraph the specific mechanism by which earthworms influence organic matter turnover will be addressed, using the theory of aggregate formation developed by Oades (1984) and Elliott and Coleman (1988) and further illustrated by Beare et al. (1994b). These authors did not consider the role of earthworms in the process. However, I think that for the Lovinkhoeve situation and for the experiment described by Beare et al. earthworms are the main agents that initialise the aggregation processes. The aggregation processes as influenced by earthworm activity presumably lead to increased C-retention capacity of soils.

According to Tisdall and Oades (1982), smaller particles are held together to form macro-aggregates by temporary (roots and fungal hyphae) and transient (polysaccharides) binding agents. Oades (1984) and Elliott and Coleman (1988) postulated that conditions within macro-aggregates are favourable for the formation of new micro-aggregates. The organic particles could become coated by bacterial mucigels and during ageing become more and more encrusted by clay particles, and thus become less available for microbial attack. Beare et al. (1994b) indeed gave some strong indications, that in the no-tillage (NT) soil with high aggregate stability and many macro-aggregates, more micro-aggregates of the 106-250 μm were formed, that contained relatively unprocessed organic material, than in the conventional tilled (CT) soil. My hypothesis is that earthworm activity offers ideal conditions for the processes of macro- and micro-aggregate formation.

First, the macro-aggregates formed by earthworm activity are enriched in organic debris in a form that could act as temporary binding agents, as described by Shipitalo and Protz (1988 and 1989). Earthworms process large amounts of soil and particulate matter: in a study by Parmelee et al. (1990) the suppression of earthworm by carbofuran resulted in higher standing stocks of unprocessed particulate organic matter in the no-tillage treatment. The organic matter of the conventional tillage treatment was much less affected, presumably because earthworm numbers already were much lower before carbofuran application. Also in the Lovinkhoeve soil the macro-aggregates had a higher C-content in the integrated soil (chapter 7). This relative enrichment would not be expected if aggregates would be formed by the same mechanisms (not involving earthworms) in all fields, so we concluded that earthworms caused this increase in C. Also Ketterings et al. (in press) found enhanced incorporation of organic matter into the stable aggregates fraction with increased earthworm activity.

The second step in the aggregation process that is stimulated by earthworm activity is the formation of new micro-aggregates within casts. This happens when the fragments that connect different parts within the macro-aggregate decompose at their surface, so that a mucigel of polysaccharides is formed, to which clay particles can adhere. Anaerobic conditions will further enhance the stabilisation of the new micro-aggregates. As shown in chapter 6, in fresh wet earthworm casts all conditions for such micro-aggregate formation are met: microbial activity is high, and at least part of the clay present is able to move and rearrange around the decomposing fragments. Anaerobic conditions are likely to occur in macro-aggregates (Tiedje, cited by Elliott and Coleman 1988). Rappoldt and Marinissen (unpub results) found that in fresh casts of *Aporrectodea caliginosa* anaerobic conditions developed if casts had a radius larger than 2 to 3 mm.

Ample evidence for the aggregate formation processes in earthworm amended macro-aggregates can be derived from a combination of the results of chapter 7 and those of Beare et al. (1994a and b). In chapter 7, in crushed stable macro-aggregates from all fields the same amounts of C per g C initially present were mineralised. I hypothesise, following Elliott (1986), that this is because it is exactly the same type of material, namely the organic particles that serve as temporary binding agents. If the mineralisation data of Beare et al (1994a) are expressed per unit of C originally present (Beare et al. 1994b), also their crushed (wet-sieving) > 2mm stable aggregates from both no-tillage (NT) and conventional tillage (CT) treatments decomposed at exactly the same rate. Their way of sampling stable aggregates by wet sieving closely resembled our methods, so I think these are very comparable results. Therefore, in both studies, stability of aggregates is linked to the same type of material, even in fields with no or reduced earthworm numbers. However, in both studies, much more stable aggregates occurred in the fields highest in earthworm numbers. Also, stable aggregates formed by earthworms differed in characteristics from stable

aggregates formed otherwise, because more micro-aggregates are formed inside these earthworm amended macro-aggregates. This explains why in the fields of Beare et al., decomposition was much lower in intact than in crushed macro-aggregates of NT (high earthworm numbers). However, because no such micro-aggregates were present in CT (low earthworm numbers) decomposition between crushed and intact aggregates of CT was the same. Crushing can very well be thought to break the aggregates along the organic fragments. In this way, crushing will expose also the fragments in newly formed micro-aggregates in the NT macro-aggregates to decomposition. The presence of newly formed micro-aggregates also explains why the stable aggregates sampled by wet sieving from the NT had lower dispersion than those from CT.

At the Lovinkhoeve, we did not measure decomposition in intact aggregates, nor did we assess the dispersion-index of stable aggregates. However, in our experiments, the effect of sonication was most pronounced in stable aggregates from int-A. Before sonication, no significant differences between mineralisation in stable and unstable aggregates occurred. After sonication, stable aggregates from IntA mineralised more than unstable aggregates (chapter 7).

Beare et al. (1994b) found that the micro-aggregate formation, which to my point of view should be ascribed to favourable conditions in earthworm casts, led to high organic matter retention capacity in NT. Also in the Lovinkhoeve soil earthworm activity led to higher retention capacity in the soil. In the Conv-A fields, where earthworm numbers had declined strongly since 1985, organic matter content of the soil decreased remarkably, even though the annual C-addition was only slightly lower than in field Int-A (Fig. 8.3).

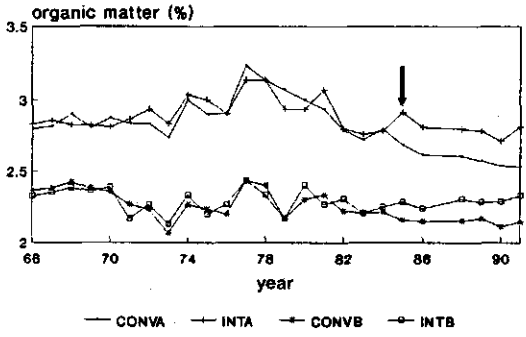

Fig. 8.3 Organic matter dynamics over 25 years at Lovinkhoeve experimental farm

Earthworm-formed stable aggregates may persist longer than those formed by other aggregation processes, but in temperate areas they certainly do not persist much longer than a few months. Aggregate stability soon declines when earthworm numbers are reduced. Because it is unlikely that earthworms would affect the stability of aggregates after they have been formed, the decline of an individual aggregate will also take place in the presence of earthworms, even though they continuously form new aggregates. The material not yet protected in newly formed micro-aggregates becomes available for mineralisation when a macro-aggregate falls apart. Stable micro-aggregates are mainly reported to contain highly processed material (Elliott 1986), while micro-aggregates

formed in earthworm casts seem to contain less processed organics. If the micro-aggregates formed within casts would have a shorter turnover time than other micro-aggregates, this might explain the higher mineralisation rates per unit of C from grasslands that are ploughed than from permanent arable land, or from Int-A when compared to Conv-A (Van Faassen and Lebbink 1994).

The process of aggregate formation and stabilisation can also take place without earthworms. The field situation that inspired Elliott to his theory was situated in a relatively dry area, where earthworms will not occur in high numbers. The relatively strong dry-wet cycles that prevail in those grasslands might act in the same way. Also, micro-arthropods might play a role in micro-aggregate formation in those soils (Brussaard and Juma, in press). However, my conviction is that when earthworms are present in the high numbers as reported in this thesis and in Parmelee et al. (1990), they quantitatively are far the most important motor behind the processes of aggregate formation and stabilisation, and subsequently organic matter retention.

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Samenvatting en conclusies

Regenwormen kunnen qua biomassa een belangrijke component van het bodemleven vormen. Als zij aanwezig zijn, leveren zij een belangrijke bijdrage aan de vorming van de bodemstructuur en aan de dynamiek van organische stof omzettingen. In natuurlijke systemen komen regenwormen vooral voor in relatief nutrient-rijke systemen, waarin ook aan hun eisen wat betreft vochtvoorziening wordt voldaan. In rijke ecosystemen is ook het strooisel van een goede kwaliteit, een belangrijke eis die wormen aan hun omgeving stellen. Juist de rijkere bodems zijn vaak in gebruik als landbouwgrond, en regenwormen kunnen dan ook in principe een belangrijke rol in agro-ecosystemen spelen. Er zijn echter veel factoren, zoals grondbewerking, gebruik van pesticiden en het toevoegen van veel of juist weinig voedingsstoffen in organische vorm, die de groeimogelijkheden van regenwormen kunnen beinvloeden. Vanuit landbouwkundig oogpunt is inzicht in het functioneren van saprotrofe voedselwebben belangrijk om te kunnen komen tot optimaliseren van mineralisatie-processen. Daarom werd op de Lovinkhoeve te Marknesse het 'Dutch Programme on Soil Ecology of Arable Farming Systems' opgestart. Een van de deelprojecten, waarover in deze thesis verslag word gedaan, had als eerste doel om de invloed van landbouwkundige ingrepen op regenworm populaties vast te stellen, maar daarnaast was ook informatie gewenst over de rol die regenwormen spelen in de bodemstructuurvorming en de afbraak van organische stof.

Het Lovinkhoeve project was opgezet als een vergelijking tussen twee landbouwsystemen: een 'conventioneel' en een zogenaamd 'geintegreerd' systeem. Het geintegreerde systeem beoogde biologische principes te integreren in een verder conventionele bedrijfsvoering. Beide systemen bestonden uit een 4-jarige rotatie van suikerbiet, zomergerst, aardappelen en wintertarwe. In het geintegreerde systeem werd gestreefd naar een zelfde verhouding tussen kosten en opbrengst als in het conventionele systeem, maar bij een lagere nutrienten input. Er werd geen gebruik gemaakt van grondontsmetting, het herbiciden-gebruik was gereduceerd en de grondbewerkings diepte was 15 cm in plaats van de gebruikelijke 25 cm. De nutrienten werden in geintegreerd voor een groter deel dan in gangbaar in organische vorm toegevoegd. De twee systemen werden uitgelegd op een oude proef, waarbij een deel al 35 jaar bij een overigens gelijke bedrijfsvoering veel meer organische bemesting had ontvangen dan de andere helft. De lay-out van de proef leverde vier combinaties van oud en nieuw management op. Naast het

management-experiment waren op de Lovinkhoeve nog andere proefvelden aanwezig, waarin steeds een enkel aspect van het management, zoals de grondbewerking of de bemestingsvorm al enige jaren verschillend was.

Regenwormen zijn relatief langzame dieren, zowel in hun voortbeweging als in hun groei en voortplanting. Dit betekent, dat verstoringen een grote invloed kunnen hebben op hun populatie-omvang, omdat er tijd nodig is voor herstel door hergroei van de populatie uit individuen die de verstoring overleefd hebben. Als er tijdens die hergroei nieuwe calamiteiten optreden, kan binnen een paar maanden de populatie tot bijna nul zijn gereduceerd.

Niet alleen reproduktie, maar ook herkolonisatie vanuit ongestoorde gebieden kan bijdragen tot het herstel van populaties. Gezien de lage snelheid van verplaatsen spelen bij (her)kolonisatie passieve verspreidingsmechanismen van wormen een belangrijke rol. Dit bleek uit onderzoek, waarbij met behulp van in de bodem gebrachtte kraaltjes een schatting werd gemaakt van de passieve verspreiding van regenwormen kokons via grond aan tractorwielen. De passieve verspreiding had een grote positieve invloed op de snelheid van koloniseren door regenwormen. Een bevestiging van dit passieve mechanisme werd gevonden in het versreidingspatroon na 1 jaar kolonisatie van wormen vauit het organisch stof-rijke deel van de Lovinkhoeve, naar het organische stof arme deel van de proef waar geintegreerd management had geleid tot betere condities voor wormen. In de rijsporen van de tractoren was de verspreiding verder gevorderd dan tussen de sporen, en de nieuwe populatie bestond alleen uit juvenielen en kokons.

Voor het succes van de kolonisatie zijn niet alleen verspreidingsmechanismen van belang, maar ook de groeicondities voor wormen. Dit werd duidelijk uit modelonderzoek. De parameters die van belang waren bij kolonisatie waren leeftijds karakteristiek van de voortplanting en overleving van de soort. Doordat goede voedselcondities en weinig verstoringen binnen kortere tijd tot een hogere coconproductie leiden, kan de organische stoftoevoer van grote invloed zijn op de kolonisatie mogelijkheden van regenwormen. Dit bleek onder andere uit het ontbreken van regenwormen in het organische stof arme deel van het Lovinkhoeve experiment, waar wormen waarschijnlijk niet hadden kunnen coloniseren door de slechte voedselcondities.

De resultaten van veldbemonsteringen op de Lovinkhoeve wezen op een grote invloed van het landbouwsysteem op regenworm populaties. Al een klein aantal jaren na het instellen van conventioneel management de aanwezige regenwormpopulaties bijna geheel verdwenen waren. De invloed van de grondontsmetting tegen nematoden werd als belangrijke oorzaak gezien. Daarnaast speelde waarschijnlijk ook de voedselvoorziening van de regenwormen een rol. In het conventionele systeem was de kwaliteit van de organische stoffen die werden toegevoegd slechter omdat ze minder stikstof bevatten. Dit directe effect

op regenwormen werd mogelijk nog versterkt doordat in geintegreerd grotere populaties van microben en protozoen voorkwamen, die een belangrijke voedselbron voor regenwormen vormen. De diepere grondbewerking in conventioneel speelde waarschijnlijk een minder belangrijke rol in de reductie van de regenworm populaties, aangezien de akkers een ecologisch gezien onvolledige groep vormden. De soorten die aanwezig waren, voornamelijk *Aporrectodea caliginosa*, *Lumbricus rubellus* en in mindere mate *Aporrectodea rosea*, zijn niet zo gevoelig voor ploegen. Ze leven in de bovenste bodemlagen, en vinden hun voedsel gedeeltelijk in de bodem. Door het ploegen kunnen ze wel beschadigen, maar dat lot bleek maar ongeveer een kwart van de populatie te treffen, en daarvan zou een deel nog kunnen herstellen van hun verwondingen. Het ploegen brengt meer organische resten in de bodem, wat weer een stimulans voor deze wormsoorten zou kunnen zijn. De soorten die ontbraken zijn zogenaamde anecique wormen, die diepe permanente gangen graven en hun voedsel zoeken op het bodemoppervlak. Deze wormen kunnen alleen goed leven als grondbewerking geheel ontbreekt, iets wat in rotaties met aardappelen en suikerbieten onmogelijk is.

Naast de menselijke ingrepen spelen in agro-ecosystemen ook natuurlijke stressfactoren een rol, zoals droogte tijdens zomermaanden of vorstperioden in de winter. Ook deze factoren speelden een rol in de levenscyclus van de regenwormen in de Lovinkhoeve, zoals bleek uit een canonische ordinatie analyse van de veldgegevens. De twee belangrijkste soorten bleken een verschillende levensstategie te hebben. In winters trekt *A. caliginosa* veel meer dan *L. rubellus* naar diepere bodemlagen. *L. rubellus* adulten en juvenielen overleven strenge winters dan minder goed dan *A. caliginosa*, maar de relatief grote afzet van cocons stelt de overleving zeker. In het voorjaar komen eerst de juveniele *A.caliginosa* naar boven, en dan de adulten, die van dieper komen. *A. caliginosa* overleeft droge zomers ook beter dan *L. rubellus*.

De regenwormen hadden invloed op verschillende aspecten van de bodemstructuur. De stabiliteit van macro-aggregaten was gerelateerd aan de actuele regenworm activiteit. Zodra de regenwormen uit het systeem verdwijnen, neemt de aggregaatstabiliteit snel af. De stabiliteit in macro-aggregaten wordt veroorzaakt door de aanwezigheid van kleine partikels organische stof, die de samenstellende deeltjes binnen het aggregaat bij elkaar houden. De stabiele aggregaten zijn daardoor rijker aan organische stof dan niet-stabiele aggregaten. De organische stof in stabiele aggregaten is in principe goed afbreekbaar, en daardoor is de levensduur van stabiele aggregaten dan ook beperkt. Stabiele aggregaten komen ook voor zonder de tussenkomst van wormen. Echter, bij aanwezigheid van wormen worden veel meer stabiele aggregaten gevormd dan zonder wormen. Hierdoor wordt een groter deel van de in de bodem aanwezige organische stof geconcentreerd in stabiele macro-aggregaten. Deze opslag biedt een zekere bescherming aan de organische stof, omdat er zuurstof gebrek

in het aggregaat kan optreden. De stabiele aggregaten die door wormen worden gevormd beschermen de erin opgeslagen organische stof echter sterker dan op andere wijze ontstane stabiele aggregaten. Door het hoge vochtgehalte van verse wormexcrementen, in combinatie met de hoge microbiele activiteit wordt de vorming van micro-aggregaten bevorderd. In de micro-aggregaten wordt de organische stof beschermd door een kleilaagje. Door de aanwezigheid van de micro-aggregaten blijven door wormen gevormde macro-aggregaten waarschijnlijk langer intact, maar zelfs als ze uit elkaar vallen vormen de overblijvende micro-aggregaten nog steeds een bescherming tegen afbraak. Hierdoor is een grond met veel wormen beter in staat om de toegevoegde organische stof in de bodem vast te houden. De organische stof die opgeslagen zit is echter van een goede kwaliteit, en heeft dus bij vrijkomen een goed mineralisatie-potentieel.

De resultaten van dit proefschrift wijzen erop, dat, in bodems waar ze voorkomen, wormen de belangrijkste factor zijn in de capaciteit van de bodem om organische stof vast te houden. Ook de gevoeligheid voor verslemping is in het algemeen veel lager in aanwezigheid van wormen. Dit leidt tot betere zaaibedcondities en een afname van het aantal macro-porien dat verstopt raakt door intern transport van klei. Voor de instand-houding van het organische stofniveau en stabiliteit is blijvende activiteit van de regenwormen nodig. Aangezien het mogelijk is, om met landbouwkundige ingrepen zoals bv grondontsmetting de wormenpopulatie uit te roeien of te reduceren, dienen dergelijke maatregelen alleen met grote zorgvuldigheid te worden toegepast.

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Het proefschrift is af! Daarmee is de tijd gekomen om ook al degenen die direct of indirect hebben bijgedragen aan de totstandkoming ervan, nog eens te bedanken en in het zonnetje te zetten.

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Curriculum vitae

Joke Marinissen werd geboren op 27 oktober 1957 te Amersfoort. Na het behalen van het VWO-diploma aan het Corderius College te Amersfoort begon zij haar studie biologie in september 1976 aan de Rijks Universiteit te Utrecht, waar zij in juli 1979 haar kandidaatsdiploma in ontvangst nam. Omdat zij haar biologische kennis in dienst wilde stellen van het duurzaam maken van de landbouw, zette zij haar studie voort aan de Landbouw Universiteit te Wageningen, waar zij in juni 1983 haar doctoraalsbul haalde met de vakken Dieroecologie, Theoretische Productie Ecologie, Bodemverontreiniging en Bodemkunde & Geologie. De praktijktijd Dieroecologie, doorgebracht bij de Universiteit van Uppsala, Zweden, had haar hart warm laten lopen voor het onderzoek.

Op 9 januari 1984 trad zij in dienst van de LUW bij de vakgroep Bodemkunde en Geologie als toegevoegd docent voor halve dagen. In dat kader gaf zij inhoud aan het nieuw geïntroduceerde onderwijs in de bodembiologie. Op 8 october 1984 kreeg zij een aanvullende half-time vaste baan bij dezelfde vakgroep als toegevoegd onderzoeker. Haar eerste taak was het opzetten van een onderzoeksproject naar het functioneren van potwormen in agro-ecosystemen (in het kader van het Lovinkhoeve project). In 1986 begon zij onderzoek naar regenwormpopulaties in het Lovinkhoeve project, het feitelijke begin van haar promotiewerk. Vanaf 1987 kwam daar onderzoek naar aggregaatstabiliteit bij, wat zich heeft ontwikkeld tot een tweede belangrijke poot van haar proefschrift.

Tijdens haar onderzoek werkte zij samen met Tony Dexter en Malcolm Oades van het Waite Agricultural Institute en Ralph Foster van de CSIRO te Adelaide, Australië (september 1988 tot februari 1989). Daarnaast waren er wederzijdse werkbezoeken met John Blair van de Ohio State University, USA (october 1990 en 1991). Samen met anderen deed zij ook onderzoek naar de invloed van regenwormen op andere aspecten dan aggregaatvorming, zoals N-mineralisatie en overleving van geïntroduceerde bacteriën.

Van 1989 tot 1992 was zij lid van het projectteam dat het Lovinkhoeve project leidde en nam deel aan de twee-wekelijkse bijeenkomsten te Haren, waarin het onderzoek naar trofische interacties in de bodem gezamenlijk vormgegeven en uitgewerkt werd. Daarnaast fungeerde zij voor velen binnen en buiten de LUW als vraagbaak over regenwormen.

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"In another apartment, I was highly pleased with a projector who had found a device of plowing the ground with hogs, to save the charges of ploughs, cattle and labour. The method is this: in an acre of ground you bury, at six inches distances, and eight deep, a quantity of acorns, dates, chestnuts, and other masts, or vegetables, whereof these animals are fondest: then you drive six hundred, or more of them, into the field, where, in a few days, they will root up the whole ground in search for their food, and make it fit for sowing; it is true, upon experiment, they found the charge and trouble very great, and they had little or no crop. However, it is not doubted that this invention may be capable of great improvement."

Jonathan Swift: Gulliver's Travels, a voyage to Laputa, etc.